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REVIEW ARTICLE

The genomic landscape of lung adenocarcinoma—insights towards personalized medicine

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Abstract
Lung neoplasms are the main source of death around the world. Non-small cell lung cancer (NSCLC) constitutes over 80% of all lung malignancies and the majority of patients present advanced disease at beginning. Risk factors for creating NSCLC have been recognized, with cigarette smoking being a major consideration along with other environmental and hereditary risk factors. Depending on the staging of lung disease, patients are acceptable for certain treatments such as surgery, radiation, chemotherapy as well as targeted therapy. Lung malignancy has a poor prognosis, over half of the individuals determined to have lung cancer die within one year and their survival rate is less than 18%. However, in the last decade, different oncogenic modifications have been found and each of them represents a potential target. With the improvement in genetics and biomarker testing, distinct mutations have been identified to better target treatment for lung cancer patients. There is still need to identify the new potential targets for the better survival rate. With the help of NGS analysis among lung adenocarcinoma patients through bioinformatics tools, novel genetic alterations can be identified to discover future potential targets towards personalized medicine. In this review, we highlight the key driver oncogenic gene mutations and fusions identified in lung cancer.

Keywords Non-small cell lung cancer (NSCLC) · Risk factors · Mutation · Malignancy · Treatment · Biomarkers

Introduction

Lung cancer is amongst the most deadly cancers for both men and women (Siegel et al. 2014). Its death rate goes beyond that of the three most common cancers i.e. (colon, breast and pancreatic) (Bresalier et al. 2015). Over 50% of patients diagnosed with lung cancer die within one year of diagnosis and the 5-year survival rate is around 17.8% (Siegel et al. 2014). In recent years, personalized medicine has begun to bring new hope to people with lung cancer, especially in Non-small cell lung cancer (NSCLC) (Marieke et al. 2018). Personalized medicine has the potential to tailor the therapy with the best response and highest safety margin to ensure better patient care. By enabling each patient to receive earlier diagnoses, risk assessments, and optimal treatments, Personalized medicine holds promise for improving health care while also lowering costs (Celine et al. 2017).

Types of lung cancer

Among two subtypes of lung cancer, small cell lung cancer (SCLC) accounts for 15% and non-small lung cancer (NSLC) accounts for 85% of all lung cancers, respectively. NSCLC is further categorized into three types: (Sher et al. 2008).

Squamous cell carcinoma contains (25–30%), Adenocarcinoma (40%), large cell carcinoma (5–10%) of all lung cancer cases.

Squamous cell carcinoma

It emerges from early forms of squamous cells in the airway epithelial cells in the bronchial tubes in the center of the lungs. This type of NSCLC is firmly correlated with cigarette smoking (Kenfield et al. 2008).

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Adenocarcinoma

It is the most well-known kind of lung malignant growth in smokers and non-smokers (Noguchi et al. 1995). It tends to occur in the periphery of the lung, which might be due to the addition of filters in cigarettes preventing large particles from entering the lungs (Couraud et al. 2012). Compared to other types of lung cancer, adenocarcinoma tends to grow slower and has a greater chance of being found before it has spread outside of the lungs (Travis et al. 1995).

Large cell carcinoma

This sort of carcinoma demonstrates no proof of Squamous or glandular development. Large cell carcinoma regularly starts in focal piece of the lungs, and sometimes into nearby lymph nodes and into chest walls (Musca et al. 1997). Large cell carcinoma tumors are strongly associated with smoking (Stellman et al. 1997).

Risk factors

Smoking is the main hazard factor for lung diseases. At the point when cigarettes turned into the real tobacco item fabricated during the 1900s, lung malignant growth turned out to be progressively common (Bresalier et al. 2015). Smoking leads to at least 80% of lung cancer deaths (Hecht 1999).

As indicated by the U.S. top health spokesperson, living with a smoker can expand a non- smoker's possibility of creating lung malignant growth by around 20–30%, and this is also known as passive smoking (Cherrie 2019). Risk factors which cause non-small cell lung cancer are given below in Table 1.

Stages in (NSCLC)

The stages of NSCLC are identified on a combination of various factors, including:

i. Size and location of the tumor.
ii. Whether the tumor is spread to the lymph node or other parts of the body (Howington et al. 2013).
iii. There are five stages for NSCLC: stages 0 (zero) and stages I through IV.

Stage 0

Known as in situ disease, which means the cancer is "in place" and has not grown into nearby tissues or has not spread outside the lungs (Chheang and Brown 2013).

Table 1 Risk factors which causes Non-small cell lung cancer (NSCLC)

Risk factors	Effecting	Death rate
Radon (linked with mine workers, natural uranium depositors, and residential radon exposures) (Krewski et al. 2005; Darby et al. 2005)	Lungs	21,000 per year
Asbestos (used in commercial and industrial projects) (Hodgson and Darnton 2000; Stayner et al. 2008)	Lining of the lungs and chest wall	0.7% (16,500 per year)
Arsenic and Arsenic compounds (antifungal, Outdoor wood preservatives, insecticides, herbicides, etc.) (van Loon et al. 1997)	Skin, lungs, bladder and kidney	5.3% lung cancer rate increases (100,000) per year
Beryllium and Beryllium oxide (X-ray and radiation technology, etc.), inhaled chemicals including cadmium, silica, vinyl chloride, nickel compounds, chromium compounds, coal products, mustard gas, and chloromethyl esters and diesel exhaust (Spyratos et al. 2013)	Lungs, liver, kidneys, heart, nervous system and the lymphatic system	1–10% increase
Air pollution (In big cities and other areas with traffic congestion, long term and accumulated exposure to air pollution, including emissions composed of polycyclic aromatic hydrocarbons (Lindeman et al. 2013)	Lungs, brain, nerves and kidney	8% increase
Certain genes and chromosomes (Carriers of *TP53* germline sequence variations who also smoke are more than 3 times more likely to develop lung cancer than nonsmokers, and also on chromosome 15 associated with lung cancer, where marker contains three genes for subunits of the nicotine acetylcholine receptor (Hwang et al. 2003; Thorgeirsson et al. 2008; Amos et al. 2008)	Lungs	People with one copy of the marker have a 30% increased risk, and with two copies have an increased risk of 70–80%

a. *Stage I* It is a small tumor which has not spread to any lymph nodes, making it possible for a surgeon to completely remove it. It is divided into two substages based on the size of the tumor:

    i. Stage IA tumors are 3 cm (cm) or less in size. Stage IA tumor may be further divided into IA1. IA2, or IA3 based on the size of the tumor.
    ii. Stage IB tumors are more than 3–4 cm or less in size, which is grown into the main airway but not the area where the windpipe (trachea) divides into the left and right bronchi, and caused a collapsed lung or it has blocked a bronchus and caused an inflammation of the lung tissues (obstructive pneumonitis) in part or all of the lung (Carson and Finley 2011).

b. *Stage II* It is also divided into two sub stages:

    i. Stage IIA cancer describes a tumor larger than 4–5 cm or lesser in size which has not spread to the nearby lymph nodes.
    ii. Stage IIB lung cancer describes a tumor that is 5 cm or lesser in size which has spread to the lymph nodes. A stage IIB cancer can also be a tumor more than 5 cm wide which has not spread to the lymph nodes. The tumor has grown into the outer membrane covering the lungs (called the parietal pleura), the chest wall, the main nerve that runs to the diaphragm (called the phrenic nerve) or the outer membrane covering the heart (called the parietal pericardium) (Spiro and Goldstraw 1984).

c. *Stage III* The tumor in the lung is 5 cm or smaller and it has spread to lymph nodes beside the windpipe on the same side of the body as the tumor, or to lymph nodes below the area where the windpipe divides into the left and right bronchi, or both (Zappa and Mousa 2016).

    i. Stage IIIA: The tumor in the lung is 5 cm or smaller and it has spread to lymph nodes beside the windpipe on the same side of the body as the tumor, or to lymph nodes below the area where the windpipe divides into the left and right bronchi (Scott et al. 2007).
    ii. Stage IIIB: The tumor is 5 cm or smaller and the cancer has spread to the lymph nodes on the opposite side of the windpipe or lung or to lymph nodes in the lower part of the neck, or the tumor is larger than 5 cm or there are 1 or more other tumors in the same lung. The cancer has also spread to lymph nodes beside the windpipe on the same side of the body as the tumors, or to lymph nodes below the area where the windpipe divides into the left and right bronchi, or both.
    iii. Stage IIIC: The tumor is larger than 5 cm or there is more than 1 tumor in a different lobe of the lung. Cancer has also spread to the lymph nodes on the opposite side of the windpipe or lung or to lymph nodes in the lower part of the neck (Spiro et al. 1984).

d. *Stage IV* The cancer has spread to other parts of the body (called distant metastasis). This is also called metastatic non-small cell lung cancer.

    i. Stage IVA cancer has spread within the chest and/or has spread to 1 area outside of the chest.
    ii. Stage IVB cancer has spread and there are 2 or more tumors growing outside of the chest (Howington et al. 2013).

Current treatment options

Surgery

Patients who have stage I, II, and IIIA NSCLC typically undergo surgery to remove the tumor if the tumor is found to be resectable and the patient is able to tolerate surgery. Surgeons may remove a lobe or section of the lung which contains the tumor. To decide whether the tumor is resectable, imaging studies and biopsies are finished just as an assessment of patient variables to decide operability. Currently, many surgeons utilize video-assisted thoracoscopic surgery (VATS), where a small incision is made in the chest and a thorascopic is inserted. A lobe can be removed via the scope through this small incision so that a larger incision does not have to be made (Howington et al. 2013).

Adjuvant

Adjuvant therapy may include radiation, chemotherapy, and targeted therapy. Patients with stage IIB and IIIA NSCLC usually receive chemotherapy after surgery to kill any remaining cancer cells in order to prolong survival (Collaborative Group. Bmj. 1995).

Chemotherapy

Approximately there are 40% of newly diagnosed lung cancer patients who have reached stage IV. The goal is to improve their survival and reduce disease related events to treat these patients. For stage IV NSCLC, cytotoxic combination chemotherapy is the first-line therapy, which might be influenced by histology, age *vs*. comorbidity, and performance status (PS) (Ramalingam and Belani 2008). Patients with performance status and their respective treatments are given below in the Table 2

Radiotherapy

It is a type of cancer treatment that uses beams of intense energy to kill DNA of cancer cells. Radiation therapy damages cells by destroying the genetic material that controls how cells grow and divide. Radiotherapy also can be part of palliative care to improve quality of life in NSCLC patients who do not respond to surgery or chemotherapy (Amini et al. 2014).

A technique called stereotactic body radiation therapy (SBRT) is used for early-stage NSCLC patients who have a single small nodule in the lung without any metastases to nearby lymph nodes (Grutters et al. 2010). The specialized equipment focuses beam of radiation on a tumor or other target. Each beam has very little effect on the tissue it passes through, but a targeted dose of radiation is delivered to the site where all the beams intersect (Fakiris et al. 2009). The high dose of radiation delivered to the affected area causes tumors to shrink and blood vessels to close off over time following treatment, robbing the tumor of its blood supply (Timmerman et al. 2010). The precision of stereotactic radiosurgery.

means there's minimal damage to the healthy surrounding tissues. In most cases, radiotherapy has a lower risk of side effects compared with other types of traditional surgery or radiation therapy. It was found that SBRT offered greater 2-year overall survival rates, lower cost and greater patient convenience (Lagerwaard et al. 2012).

Biomarker testing

In the past, patients with the same type and stage of lung cancer received the same treatment. Today, researchers know that no two persons are exactly the same; no patients' tumors are exactly the same. In some cases, doctors can use information about your tumor to help them decide whether one treatment is more likely than another to work in your case. This new approach to cancer treatment, called personalized medicine, is made possible by the discovery of biomarkers. Biomarkers are molecular characteristics of a tumor that can, in some cases, be used to help make decisions about treatment. Biomarker tests available to lung cancer patients include: (Riely et al. 2009).

The recent discovery of activating mutations in EGFR, $KRAS^{G12C}$, BRAF (V600E), and fusion genes involving ALK, ROS1, RET and many other genes has set the stage for personalized medicine for lung cancer. Patients having mutations in these biomarkers are being benefited from the development of various inhibitors with considerable improvement in tumor control and survival. Four key areas of knowledge that are essential to the development of targeted therapy, i.e.; knowing the target, knowing the biomarker, knowing the end point and knowing the mechanisms of resistance (Buuren 2015).

I. *EGFR GENE* (Epidermal Growth Factor Receptor)
II. *ALK GENE* (Anaplastic Lymphoma Kinase)
III. *KRAS GENE* (Kristen Rat Sarcoma Viral Oncogene)
IV. *Tp53 GENE* (Tumor Suppressor)
V. *BRAF GENE* (v-raf sarcoma viral oncogene serine/threonine kinase)
VI. *ROS1* Gene arrangement (ROS Proto-Oncogene 1, Receptor Tyrosine Kinase)
VII. *RET* Gene arrangement (RET Proto-Oncogene)
VIII. *PD-L1* Expression (Programmed death ligand 1)

Table 2 Patients with performance status (PS) and their treatment

Patients with PS (performance staus)	Treatment	Survival rate
0–1	It is a regimen of a platinum (cisplatin or carboplatin) plus paclitaxel, gemcitabine, docetaxel, vinorelbine, irinotecan, or pemetrexed (specific combination depends on types and frequencies of toxic effects and should be decided on an individual basis. However, adenocarcinoma patients may benefit from pemetrexed) (Kelly et al. 2001; Scagliotti et al. 2002; Schiller et al. 2002; Fossella et al., 2003)	8–10 months
2	They may need only one drug, which is typically not platinum (For chemotherapy treatment, serious adverse events should prompt a change in agents. Therapy should also be stopped if the cancer grows or if, after four treatment cycles, the disease is stable but the treatment is not shrinking the tumors) (Amini et al. 2014)	5 months
3 (Lagerwaard et al. 2012)	Pathological diagnosis (patients with PS of 3, here they could worsen their quality of life Significantly) (Pisters et al. 2007)	Less than 5 months

I. *EGFR GENE* (Epidermal Growth Factor Receptor)

Simplified schema of epidermal growth factor receptor *(EGFR)*-induced signals that regulate critical cellular functions.

Figures adapted and modified from literature search performed for relevant studies for meeting the inclusion criteria. Nine to ten relevant papers were formally reviewed for the present study.

The *EGFR* gene provides instructions for making a receptor protein called the Epidermal Growth Factor Receptor, which spans the cell membrane so that one end of the protein remains inside the cell and the other end projects from the outer surface of the cell. This positioning allows the receptor to attach (bind) to other proteins, called ligands, outside the cell and to receive signals that help the cell respond to its environment, Ligands and receptors fit together like keys into locks (Wei Li et al. 2017). Epidermal growth factor receptor binds to at least seven different ligands. The binding of a ligand to epidermal growth factor receptor allows the receptor to attach to another nearby epidermal growth factor receptor protein (dimerize), turning on (activating) the receptor complex, shows in Fig. 1. As a result, signaling pathways within the cell are triggered that promote cell growth and division (proliferation) and cell survival (Huang 2016).

After dimerization, the kinase is tagged with a marker called a phosphate group (a cluster of oxygen and phosphorus atoms) in a process called phosphorylation. Phosphorylation turns on (activates) the kinase. The activated kinase is able to transfer a phosphate group to another.

protein inside the cell, which is activated (Lindeman et al. 2013). As a result, auto phosphorylation of several tyrosine (Y) residues in the C-terminal domain of *EGFR* occurs. These include Y992, Y1045, Y1068, Y1148 and Y1173, as shown in the diagram given below. This auto phosphorylation elicits downstream activation and signaling by several other proteins that associate with the phosphorylate tyrosine through their own phosphotyrosine-binding SH2 domains (Lohinai et al. 2015). These downstream signaling proteins initiate several signal transduction cascades, principally the *MAPK, Akt* and *JNK* pathways, leading to DNA synthesis and cell proliferation. Such proteins modulate phenotypes such as cell migration, adhesion, and proliferation (Bethune et al. 2010). The kinase domain of *EGFR* can also cross-phosphorylate tyrosine residues of other receptors it is aggregated with, and can itself be activated in the manner (Sigismund et al. 2018). *EGFR* belongs to the *HER/erbB* family of receptor tyrosine kinases (RTKs), which includes *HER1 (EGFR/erbB1), HER2 (neu, erbB2), HER3 (erbB3*), and *HER4 (erbB4*). These receptors display similar molecular structures: They have an extracellular, cysteine-rich ligand-binding domain; a single α-helix transmembrane domain; a cytoplasmic TK domain (in all receptors except *HER3);* and a carboxyl-terminal signaling domain (Wells 1999).

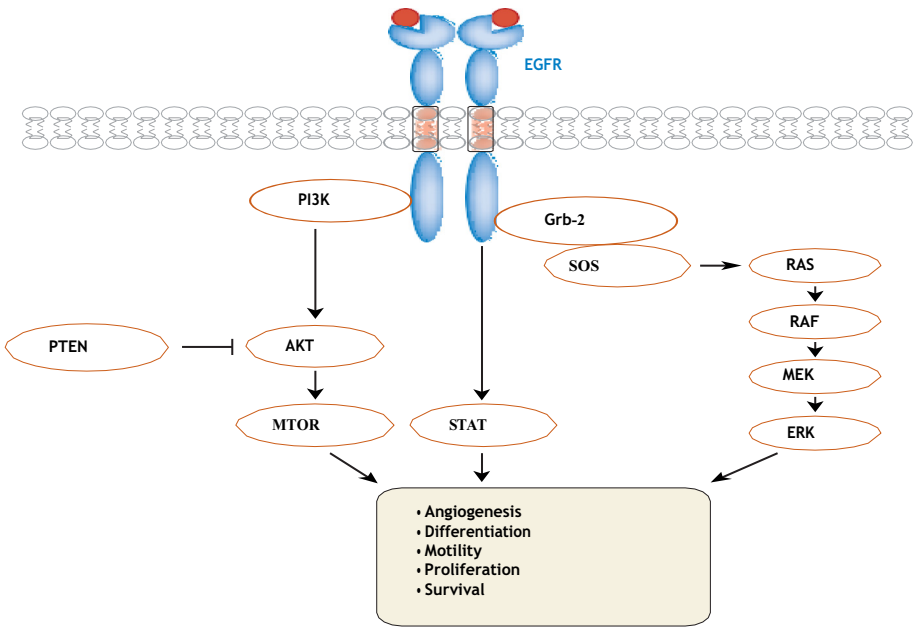
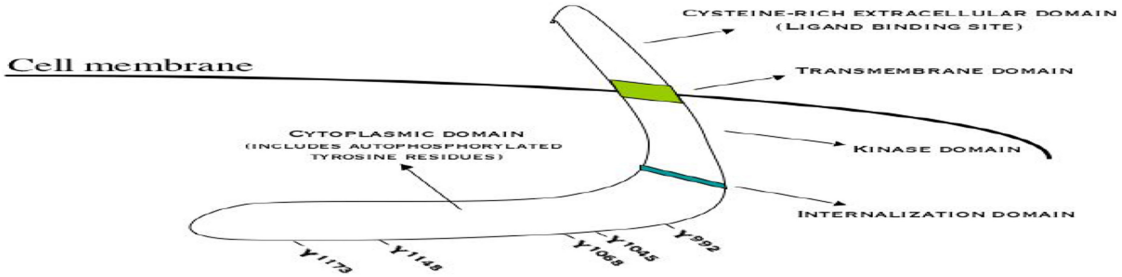


Fig. 1 Intracellular signaling is mediated mainly through the *RAS-RAF-MEK-MAPK* pathway, the *P13K-PTENT-AKT*, and signal transducer and activator of transcription. *ERK* extra cytoplasmic-regulated kinase, *Grb-2* growth factor Receptor–bound protein 2, *MAPK* mitogen-activated protein kinase, *MEK MAPK* kinase, *mTOR* mammalian target of rapamycin, *PI3K* phosphoinositide 3-kinase, *PTEN* phosphatase and tensin homolog, *RAF* v-raf murine leukemia viral oncogene homolog, *RAS* rat sarcoma viral oncogene homolog, *SOS* sister of seven less, *STAT* signal transducer and activator of transcription



At least eight mutations in the *EGFR* gene have been associated with lung cancer. Lung cancer is a disease in which certain cells in the lungs become abnormal and multiply uncontrollably to form a tumor. Lung cancer may not cause signs or symptoms in its early stages, nearly all these *EGFR* gene mutations occur during a person' s lifetime (somatic) and are present only in cancer cells (Alessandro et al. 2019). See for more information in Table 3.

Somatic mutations in the *EGFR* gene most often occur in a type of lung cancer called non-small cell lung cancer, specially a form called adenocarcinoma. These mutations are most common in people with the disease who has never smoked. Somatic *EGFR* gene mutations occur more frequently in Asian populations than in white populations, occurring in 30 to 40% of affected Asians compared to 10 to 15% of whites with lung cancer (Calabuig 2017).

Most of the somatic *EGFR* mutations that are associated with lung cancer delete genetic material in a part of the gene known as exon 19 or change DNA building blocks (nucleotides) in another region called exon 21. These gene changes result in a receptor protein that is constantly turned on (constitutively activated), even when it is not bound to a ligand. As a result, cells constantly receive signals to proliferate and survive, leading to tumor formation. When these genetic changes occur in cells in the lungs, lung cancer can develop (Alessandro et al. 2019).

Lung cancer with *EGFR* gene mutations tend to respond to treatments that specifically target the overactive epidermal growth factor receptor protein that allows cancer cells to constantly grow and divide (Toshihide et al. 2020).

II. ALK GENE (Anaplastic Lymphoma Kinase)

The Anaplastic Lymphoma Kinase (*ALK*) gene provides instructions for making a protein called *ALK* receptor tyrosine kinase, which is part of a family of proteins called receptor tyrosine kinases (RTKs). Receptor tyrosine kinases transmit signals from the cell surface into the cell through a process called signal transduction (Ardini et al. 2010). As the majority of *RTKs* (Receptor tyrosine kinase), following binding of the ligand, the full-length receptor *ALK* dimerizes, changes conformation and auto-activates its own kinase domain, which in turn phosphorylates in *trans* other *ALK* receptors on specific tyrosine amino acid (Du et al. 2018). Phosphorylated *ALK* activates multiple downstream signal transduction pathways, such as the *MAPK-ERK*(mitogen- activated protein kinases)- extracellular signal-regulated kinases), the *PI3K-AKT* (phosphoinositide 3-kinase and protein kinase B), the *PLCγ* (Phospholipase C Gamma), the *CRKL-C3G*, and the *JAK-STAT*(Janus kinase/signal transducers and activators of transcription) pathways among other (Zwaenepoel et al. 2015). These signaling pathways are important in many cellular processes such as cell growth and division (proliferation) or maturation (differentiation) which shows in Fig. 2. Most mutations of the *ALK* gene are in the form of a translocation with another partner gene leading to a fusion oncogene (Didik et al. 2020).

The fusion proteins created by these rearranged genes have functions of both *ALK* receptor tyrosine kinase and the partner protein nucleophosmin (NPM-ALK), resulting from a chromosomal translocation. The presence of the partner protein allows phosphorylation of *ALK* receptor tyrosine kinase without dimerization (Janoueix-Lerosey et al. 2008). The fusion protein and signaling pathways activated by *ALK* receptor tyrosine kinase are constitutively activated, which may abnormally increase the proliferation of immature nerve cells, leading to lung cancer formation (Arbour and Riely 2017). See for more information in Table 3.

III. *KRAS GENE* (Kristen rat sarcoma viral oncogene)

The ability of single-stranded murine sarcoma virus, Kirsten and Harvey, to transform normal mammalian genes into potent oncogenes was discovered over four decades ago. These viral oncogenes were only able to generate rat sarcomas for what they were called RAS genes, *KRAS* and *HRAS* alluding to its discoverers (Scolnick et al. 1973; Harvey 1964). The *KRAS* gene is in the Ras family of oncogenes, which also includes two other genes: *HRAS* and *NRAS* (Addissie et al. 2015). The *KRAS* (Kristen Rat Sarcoma Viral oncogene) gene provides instructions for making a protein called K-Ras that is part of a signaling pathway known as the RAS/MAPK pathway. The protein relays signals from outside the cell to the cell's

Table 3 Summary of molecular targets which are currently routinely available in non-small cell lung cancer (NSCLC)

Target	Total exon	Mutation	Chromosome location	Type of person	Personalized treatment	Frequency
EGFR	31	Exon 18 to 21 and rare exon 22 mutation Exon 19: loss of E746 to S752 i.e.; elimination of leucine-arginine-glutamate-alanine motif in the TK domain Exon 21: thymine to guanine transversion that results in an arginine for leucine substitution at amino acid 858(L858R) Exon 22: (E884K) (Huang et al. 2004; Lynch et al. 2004; Paez et al. 2004)	7P11.2	Non-smokers and only females	Targeted tyrosine kinase inhibitor therapy (erlotinib, geftinib, Afatinib, Osimertinib, Dacomitinib)	14–25%
ALK	29	small inversion within chromosome 2p between the exon 20 of the ALK (anaplastic lymphoma kinase) gene and different exons of EML4 (echinoderm microtubule-associated protein-like 4) (Sasaki et al. 2010)	2p23.2-p23.1	Never smokers or light smokers	Crizotinib (xalkori, Pfizer),	4–5%
KRAS	6	Most mutations in RAS gene affect exons 2 and 3 (Karachaliou et al. 2013)	12P12.1	Smokers	KRAS inhibitor (Sotorasib)	15–25% in all lung cancers
Tp53	12	Missense Mutations are often clustered in the 4–9 exons of the TP53 gene (Liu et al. 2014)	17p13.1	Smokers	MDMA(murine double minute 2) inhibitor	
BRAF	21	Point mutation in exon 15 (V600E) (Loupakis et al. 2016)	7q34	Current and light smokers	Vemurafenib, Sorafenib, Dabrafenib, Trametinib	2–3%
ROS1	46	ROS1 rearrangements lead to fusions of an intact ROS1 tyrosine kinase domain with partner genes, which are usually present on another chromosome	6q22.1	Light and non- smokers	Crizotinib, Ceretinib	1–2%
RET	20	KIF5B-RET is a fusion gene between KIF5B and the RET proto-oncogene caused by a Pericentric inversion of 10p11.22- q11.21. Fusion occurred between the 16th exon of KIF5B and the 12th exon of RET	10q11.21	Females, and/or never/light smokers	Cabozantinib	1–2%
PD-1/PD-L1	7	PD-1 overexpression on CD8 + 7 cells produce a reduced production of cytokines and T-cells proliferation, leads to tumor	9p241	Males, never/former smokers	Nivolumab and Pembrolizumab	27–57%

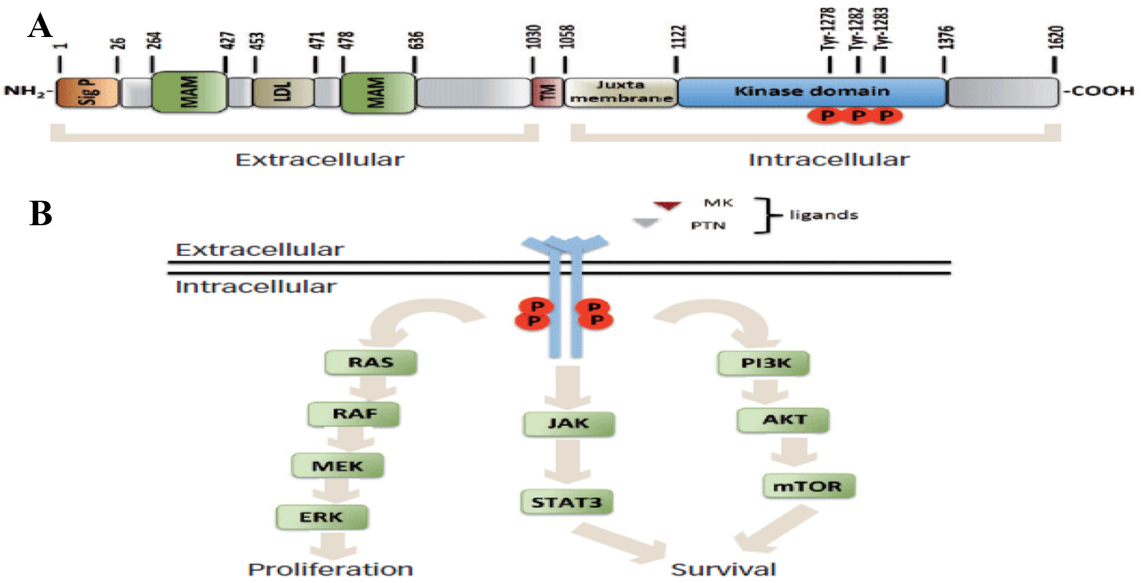


Fig. 2 The extracellular region contains a signal peptide (sigP)—MAM domain, which is an acronym derived from meprin. **A** 5 protein, and receptor protein-tyrosine phosphatase mu, and one low density lipoprotein (LDL) domain. The transmembrane domain (TM) connects the extracellular and intracellular regions. The intracellular region contains the juxtamembrane domain and the tyrosine kinase catalytic domain, which contain three tyrosine phosphorylation sites necessary for activation. **B** The binding of ligands to receptor leads to the activation of the tyrosine kinase domain and concomitant activation of multiple signaling pathways

KRAS:

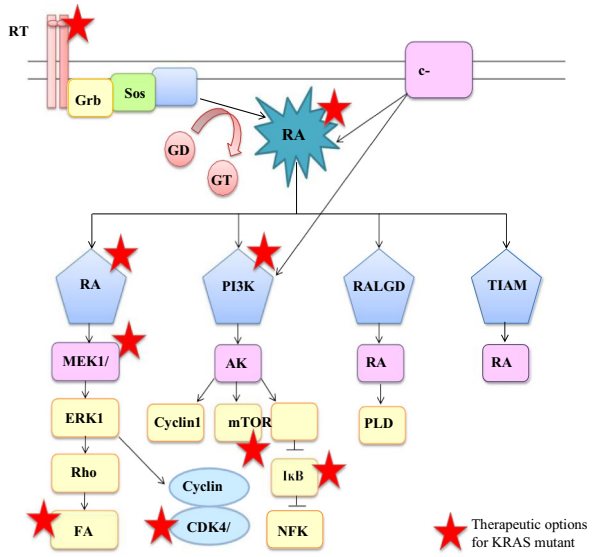


Fig. 3 **A** The downstream signaling is regulated by two alternative states of *RAS* proteins: *RAS-GTP* (active form) and *RAS-GDP* (inactive form). *RAS-GTP* complex activates several downstream signaling effectors such as the canonical *Raf-MEK-ERK*, the *P13K-AKT-mTOR* and *RalGDS-RalA/B* pathways or the *TIAM1-RAC1* pathway. **B** The exchange of GDP-GTP is regulated by additional proteins: Guanine nucleotide exchange factors (GEFs) decrease the affinity of RAS proteins for GDP and favor GTP binding that results in RAS activation, while GTPase activating proteins (GAPs) accelerate the intrinsic GTPase activity to regulate the RAS cycle

nucleus, shows in Fig. 3. These signals instruct the cell to grow and divide (proliferate) or to mature and take on specialized functions (differentiate) (Addissie et al. 2015). *KRAS* activation allows the downstream signaling mediated by EGF (Chong and Janne 2013). The *K-Ras* protein is a GTPase, which means it converts a molecule called GTP into another molecule called GDP. In this way the K-Ras protein acts like a switch that is turned on and off by the GTP and GDP molecules. To transmit signals, it must be turned on by attaching (binding) to a molecule of GTP (Carta et al. 2006). The *K-Ras* protein is turned off (inactivated) when it converts the GTP to GDP. When the protein is bound to GDP, it does not relay signals to the cell's nucleus (Castagnola and Giaretti 2005) (Figs. 4, 5, 6, 7, 8).

The *KRAS* gene belongs to a class of genes known as oncogenes. When mutated, oncogenes have the potential to cause normal cells to become cancerous (Gremer et al. 2011; Karachaliouet et al. 2013; Nava et al. 2007). These *KRAS* gene mutations are somatic, which means they are acquired during a person's lifetime and are present only in tumor cell (Schubbert et al. 2006; Prior et al. 2012). These mutations impair the GTPase activity promoting the active GTP-bound state. Generally, the G→A transition in codons 12 or 13 is dominant in *KRAS* isoform resulting in G12D or G13D mutations, followed by G→T transversions that produce G12V (Karachaliou et al. 2013; Prior et al. 2012). The most frequent mutation in *KRAS* mutant NSCLC is G12C (41%). See for more information in Table 3.

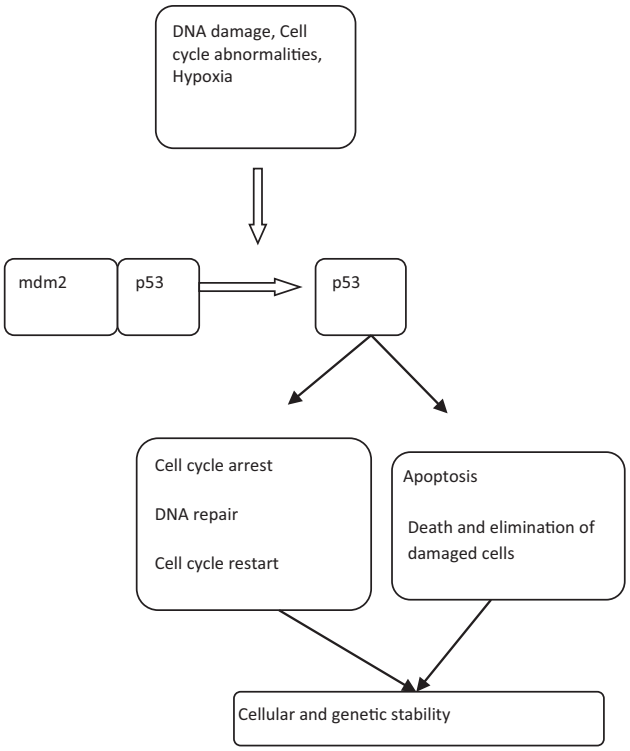


Fig. 4 *P53* pathway: In a normal cell, p53 is inactivated by its negative regulator, such as murine double minutes 2 (mdm2), MDM2 is an E3 ubiquitine which benefits the *p53* degradation via proteasome. Upon DNA damage or other stresses, various pathways will lead to the dissociation of the p53 and mdm2 complex. Once activated, *p53* will induce a cell cycle arrest to allow either repair or survival of the cell or apoptosis to discard the damaged cell

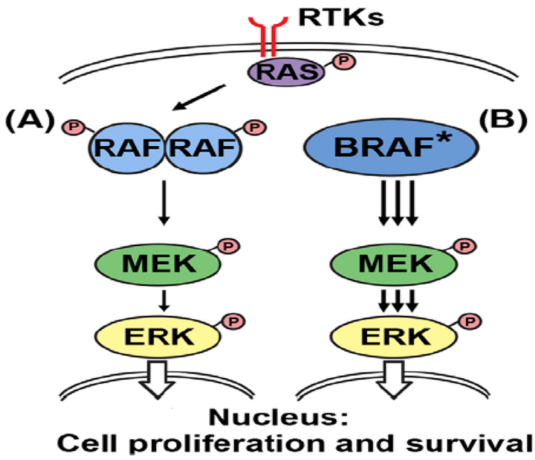
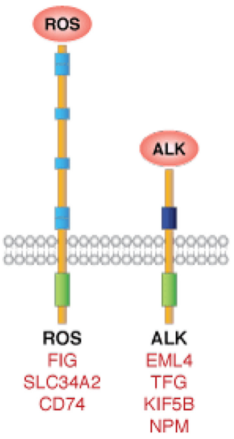


Fig. 5 **A** Under normal conditions, *RAS* activation provides the anchor for dimerization of RAF proteins (*ARAF, BRAF and CRAF).* The activated *RAF d*imer complex then activates the downstream effector molecules *MEK/ERK* resulting in expression of genes for cell proliferation and survival. **B** Activating *BRAF* mutant constitutively activates the *MAPK/ERK* pathway in the form of a monomer without the need of an activated *RAS*. RTK indicates the receptor tyrosine kinase

Fig. 6 *ALK* (Anaplastic Lymphoma Kinase) and *ROS1* (ROS proto-oncogene 1 are related receptor tyrosine kinases (RTKs). Research studies have identified *ALK* and *ROS* as mutant C- terminal fusion proteins that are expressed in a wide range of NSCLC (Jessica et al. 2017)



It has been proposed that *KRAS*-mutated tumors behave similarly to the *KRAS, EGFR* and *ALK*-native tumors with respect to sites of metastases. However, this may reflect biological heterogeneity, as it has been suggested that the type of point mutation may affect downstream signaling differently, which may translate into different clinical features (Doebele et al. 2012).

IV. Tp53 gene

The *TP53* gene provides instructions for making a protein called tumor protein p53 (or p53). This protein acts as a tumor suppressor, which means that it regulates cell division by keeping cells from growing and dividing (proliferating) too fast or in an uncontrolled way (2014). The *p53* protein is located in the nucleus of cells throughout the body, where it attaches (binds) directly to DNA. When the DNA in a cell becomes damaged by agents such as toxic chemicals, radiation, or ultraviolet (UV) rays from sunlight, this protein plays a critical role in determining whether the DNA will be repaired or the damaged cell will self-destruct (undergo apoptosis). If the DNA can be repaired, *p53* activates other genes to fix the damage (Zwaenepoel et al. 2015). If the DNA cannot be repaired, this protein prevents the cell from dividing and signals it to undergo apoptosis. By stopping cells with mutated or damaged DNA from dividing, *p53* helps prevent the development of tumors. As p53 is essential for regulating cell division, cell survival, DNA repair, apoptosis and senescence and preventing tumor formation, it has been nicknamed the "guardian of the genome" (Loyo et al. 2013; Masica et al. 2015; Olivier et al. 2010).

Cell cycle arrest and senescence are mainly mediated by a p53 downstream effector named p21, which is encoded by the gene WAF. p53 promotes DNA repair by mainly activating *GADD45* (Growth arrest and DNA damage) and *P53R2* (p53 inducible ribonucleotide reductase) genes. As a result DNA damage is repaired. In response to severe stress signals causing irreparable DNA changes, p53 usually induces genes such as *PUMA* (p53 upregulated modulator of apoptosis),

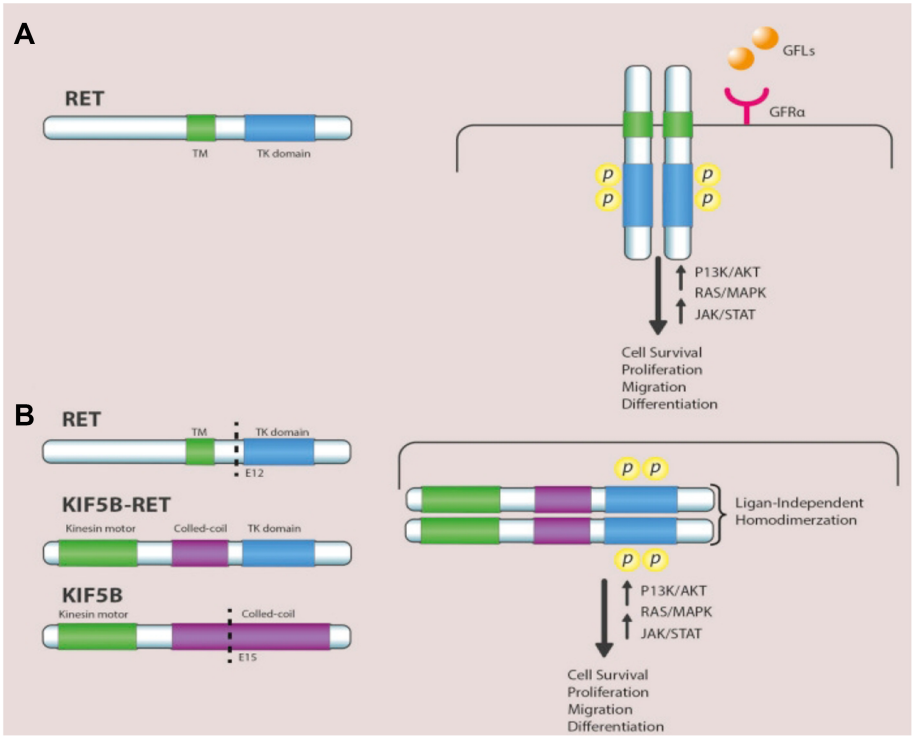


Fig. 7 Rearranged during transfection proto-oncogene gene *(RET)* receptor (**A**) and *KIF5B- RET* fusion protein (**B**) activations, with associated triggered downstream pathways. *TM* transmembrane domain, *E* exon, *TK* tyrosine kinase, *GFLs GDNF* family of ligands, *GFRα GDNF* family receptor α, *P13K* phosphoinositide 3-kinase, *MAPK* mitogen activated protein kinase, *JAK* Janus Kinase, *STAT* signal transducer and activator of transcription; *KIF5B*, kinesin family member 5B (Roberto et al. 2017). See for more information in Table 3

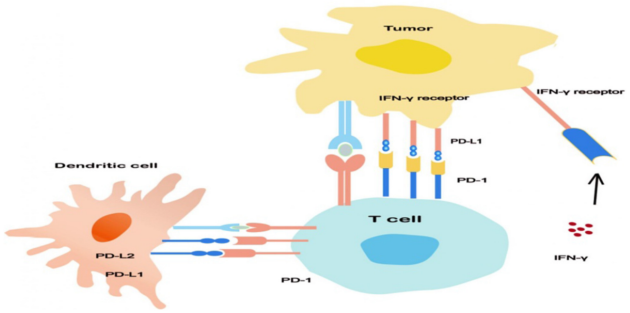


Fig. 8 Mechanism of *PD-1/PD-L1* pathway

Bax, FAS, PIG3 and killer/DRS (Perri et al. 2015; Haupt et al. 2016; Lee and McKinnon 2009). Wild type p53 is main controller of intracellular *ROS* level (*ROS* reactive oxygen species). P53 regulate the level of *NRF2* through p21. In response to severe stress, *p53* inhibits *NRF2* and allowing the *ROS* accumulation and apoptosis induction (Perri et al. 2016). See for more information in Table 3.

P53 regulate glycolysis, pentose phosphate pathway (PPP), mitochondrial oxidative phosphorylation, lipids and nucleotides metabolism and, importantly, the cell response to oxidative stress. Unlike the majority of cells, which depend on oxidative phosphorylation to provide energy, tumor cells mainly utilize glycolysis even in presence of sufficient oxygenation. This phenomenon (glycolysis in aerobic conditions) is named Warburg effect. Wild type p53 upregulates oxidative phosphorylation and downregulates glycolysis inducing its target genes such as SCO2 (synthesis of cytochrome oxidase 2) and GLS2 (mitochondrial glutaminase) which promotes oxidative phosphorylation, and TIGAR (TP53 inducible glycolysis and apoptosis) which inhibit glycolysis (Muller and Vousden 2013). Moreover, p53 block the intracellular glucose uptake inhibiting the expression of GLUT1 and GLUT4 (glucose transporter) (Jiang et al. 2011). Tumor cells strongly need a fatty acid synthesis and wild type p53 arrest fatty acid synthesis and thus block the formation of new phospholipids' membrane to support the rapid growth and division of tumour cells. Mutant p53 constitutively activates the ribonucleotide reductase (RRM2) to facilitate the conversion of ribonucleotide diphosphate to deoxy-ribonucleoside diphosphate, which is an essential step for DNA synthesis (Perri et al. 2016; Harris and Levine 2005).

Somatic mutations in the *TP53* gene have been found in nearly half of all lung cancer. Lung cancer is a disease in which certain cells in the lungs become abnormal and multiply uncontrollably to form a tumor. Signs and symptoms may not occur in early stages of the disease (Olivier et al. 2006).

Small cell lung cancers nearly always have *TP53* gene mutations; however, these mutations may also occur in non-small cell lung cancer. *P53* is often mutated in solid tumors. *TP53* gene mutations change single amino acids in *p53*, which impair the protein's function (Ruijs et al. 2010). Without functioning *p53*, cell proliferation is not regulated

effectively and DNA damage can accumulate in cells. Such cells may continue to divide in an uncontrolled way, leading to tumor growth. Additional genetic, environmental, and lifestyle factors contribute to a person's cancer risk; in lung cancer, the greatest risk factor is being a long-term tobacco smoke (Silwal-Pandit et al. 2014; Hmitou et al. 2007).

V. BRAF GENE

BRAF is a serine/threonine protein kinase that plays a crucial role in the mitogen activated protein kinase (*MAPK)/*extracellular signal-regulated kinase *(ERK*) pathway, involved in cell growth, proliferation, survival and differentiation. *MAPK/ERK* pathway includes several proteins with 4 kinase domains (*RAF, MEK, ERK)* that convey signal transduction from membrane receptors to DNA in the nucleus of the cell (89). Three different RAF isoforms, originating from three different genes, can be distinguished in mammals (*ARAF, BRAF* and *CRAF*); despite similar structure, they are not equal in ability to activate MEK substrate and their dimerization (both in terms of homodimerization and heterodimerization) is a crucial step in RAF catalytic activation (Rushworth et al. 2006).

Activating mutations in the *BRAF* gene, generally mutually exclusive from *EGFR* mutations or *ALK* rearrangements, act as an alternative oncogenic driver in NSCLC.

The *BRAF* gene belongs to a class of genes known as oncogenes. When mutated, oncogenes have the potential to cause normal cells to become cancerous (Dhomen and Marais 2007).

Somatic mutations in the *BRAF* gene are common in several types of lung cancer. Normally, the *BRAF* protein is switched on and off in response to signals that control cell growth and development. See for more information in Table 3. Somatic mutations cause the *BRAF* protein to be continuously active and to transmit messages to the nucleus even in the absence of these chemical signals (Harmon and Brown 2015). The overactive protein may contribute to the growth of cancers by allowing abnormal cells to grow and divide without external signals (Haroche et al. 2014). The V600E mutation is the most common *BRAF* gene mutation found in human cancers (Roden et al. 2014). This mutation confers two oncogenic properties to *BRAF* protein: first, it increases *BRAF* kinase domain activity; secondly, it enables *BRAF* to be active as a monomer when RAS activity is low, independently of RAS-mediated activation. The result is a hyperactivated protein that continuously activates ERK, bypassing RAS activation and ignoring ERK-dependent negative feedback (Yao et al. 2015).

VI. *ROS1* Gene Arrangement: (*ROS* Proto-Oncogene 1, Receptor Tyrosine Kinase)

ROS1 is a proto oncogene encoding a Type I integral membrane protein with receptor tyrosine kinase *(RTK)* activity. *ROS1* is a member of insulin receptor family and is involved in downstream signaling processes involved in cell growth and differentiation.

ROS1 has been described as distinct molecular type in approximately 1–2% of patients with NSCLC (Duruisseaux 2019). The *ROS1* locus is located on chromosome 6 and encodes for an orphan tyrosine kinase receptor, i.e. with no known ligand and biological function in humans. Fluorescence insitu hybridization (FISH) is considered the gold standard method for diagnosis *ROS1* rearrangements, although next generation sequencing (NGS) is rapidly gaining a role. See for more information in Table 3.

Ros1fusion pair its kinase domain with an array of partners promoting constitutive *ROS1* kinase activation. The CD74-ROS1 fusion has been reported as the most common rearrangement in NSCLC. Other fusion partners include *SDC4, EZR, SLC34A2, TPM3, LIMA, AND MSN* (Moesin). The fusion lead to constitutive activation of *ROS1* kinase that derives cellular transformation and promotes survival and proliferation through downstream signaling via *SHP-1/SHP-2, JAK/STAT, P13K/AKT/MTOR and MAPK/ERK* pathways (Giulio Rossi et al. 2017).

VII. *RET* gene arrangement (*RET* proto- oncogene)

The gene encodes a transmembrane receptor and member of the tyrosine protein kinase family of proteins. The ligands of the RET receptor belong to the glial cell line derived neurotrophic factor *(GDNF),* neurturin (*NRTN*), artemin (*ARTN),* and presepsin *(PSPN).* The *RET* receptor and its ligand form a multimeric complex that can activate the kinase domain, resulting in auto phosphorylation of the intercellular domain. Activation of *RET* protein can activate several signaling pathways, including those of mitogen activated protein kinase(MAPK), phosphoinositide 3-kinase (P13K/AKT, Rac/c-jun NH, kinase (JNK), phospholipase Cγ, and Ras/mitogen- activated protein (MAP) kinase. Normally *RET* is essential for the development of the enteric nervous system, kidney and embryogenesis of mammals, and its expression level in normal lung tissue is low (Stinchcombe 2020).

A rare genetic alteration, which is called *RET* rearrangement is detected in 1–2% of Non-small cell lung cancer (NSCLC). On the other hand, Gene rearrangements involving *RET*, have been categorized mostly extensively in papillary thyroid carcinomas, and later have been seen in other cancers, specifically in lung cancer (Justin 2013)**.**

VIII. *PD-L1* Expression (Programmed death ligand 1)

Immunotherapy, which is a novel method of lung cancer treatment, also contributes to such improvements. The latest method of immunotherapy uses monoclonal antibodies directed against the immune- checkpoint molecules such as a receptor programmed cell death *(PD-1*) or its ligand

(PD-L1). Immune- oncology (I-O) can be classified into three categories: (1) active immunity includes vaccines, cytokines, and checkpoint inhibitors; (2) passive immunity includes adoptive cell infusion and targeted monoclonal antibodies; (3) hybrid immunity are combined of active and passive methods. Among them, immune checkpoints were considered as the most promising targets, whose existence helps to terminate immune activity to guard against autoimmunity and allow for self-tolerance in normal physiological conditions. However, these immune checkpoint pathways can be tricked and hijacked by tumors to evade the attack of immunity system. Checkpoint inhibitors can reactivate the antitumor immunity delay tumor growth and lengthen the survival by blocking this pathway (Konrad et al. 2019).

PD-1 is a member of *CD28* family which is expressed in several kinds of immune cells, especially in activated *CD8+T* cells, and B cells in peripheral tissues. *PD-1* has two binding ligands, *PD-L1(B7-H1or CD274*) and *PD-L2(B7-DC* or *CD273*). *PD-L1* is expressed on activated T cells, B cells, macrophages, dendritic cells and cancer cells. The binding of *PD-1/PD-L1* suppresses the proliferation, survival of the cytotoxic T lymphocyte, induces the apoptosis process of infiltrative T cells and reduces the production of cytokines. See for more information in Table 3.

PD-1/PD-L1 pathways plays a vital role in immune escape of tumor cells. *PD-1* or *PD-L1* inhibitors block the inhibitory T-cell signaling, and reactivating the antitumor activity of *CD8+*T cells. Tumor cells escape the immune response through the upregulation of *PD-L1* to inhibit the action of T cells (Minghui et al. 2017).

Distinct oncogenes/tumor suppressor genes as well as new approaches involved in NSCLC

There are various lung adenocarcinoma oncogenes and tumor suppressor genes in the above review which have ability to therapeutically inhibit the functions of those altered genes would therefore represent significant progress in the battle against lung cancer. Apart from that, there are various other mutually/non mutually exclusively oncogenic alterations which are involved in lung adenocarcinoma i.e.; The *MET* proto-oncogene which is located on chromosome 7, where *EGFR* and hepatocyte growth factor (*HGF)* genes also reside. *MET* encodes a receptor tyrosine kinase (RTK), which binds to its natural ligand, *HGF,* also called scatter. *LKB1* gene (also known as stk11) is a tumor suppressor gene located on chromosome 19p13.3. It was discovered that *LKB1* possesses inactivating mutations in NSCLC tumors and was found to be a fairly common event. The *P1K3CA* gene encodes p110a, one of the catalytic subunits of phosphatidyl inositol 3-kinase (P13Ks), which belongs to a family of lipid kinases involved in many cellular processes, including cell growth and proliferation. *NTRK* genes encode tropomyosin receptor kinases(TRK) family proteins, which includes three members *(TRKA, TRKB,* encoded by *NTRK1, NTRK2, NTRK3* respectively). Different mechanisms can be responsible of TRK oncogenic activations, although gene fusions involving *NTRK1, NTRK2*, *NTRK3* are the most commonly observed in solid- tumors, including NSCLC (Roy et al. 2018).

The fibroblast growth factor/fibroblast growth factor receptor (FGFR/FGF) signaling pathway fundamentally regulates embryogenesis, adult tissue homeostasis, angiogenesis and wound repair. It also plays important roles in cell functions, including proliferation, differentiation, apoptosis and migration. Deregulated *FGF/FGFR* activations have been associated with a broad range of developmental disorders and cancer progression (Hong-ping Huang et al. 2015).

Immunotherapy and Liquid biopsy in NSCLC

Immunotherapy is a breakthrough treatment in oncology that uses the body's own natural defense system to fight off cancer. Some cancer cells share characteristics with healthy cells and thus the immune system cannot differentiate between the body's normal and abnormal (cancer) cells. It is believed that immunotherapy works by boosting the immune system so that it can target cancer cells and stop or slow the growth of cancer cells, by preventing cancer cells from spreading to other parts of the body, or by helping the immune system increase its effectiveness. Higher numbers of CD4 + T cells, CD8 + T cells, natural killer cells, and/or dendritic cells are associated with better patient survival (Christian Rolfo et al. 2017).

Potential markers may be found in various biological samples-e.g. blood, urine, tissues, Branchioalveolar lavage, as well as in saliva and sputum- but none have been moved to the clinical setting yet. A new approach in lung cancer detection is liquid biopsy-i.e.; the sampling and analysis of component isolated or purified from a non- solid biological tissue. Liquid biopsy refers to the detection of tumor components in body fluids, such as urine, saliva, cerebrospinal fluid and liquid cytology specimens, but primarily in blood (plasma). It is easily accessible and allows the near real-time monitoring of the cancer (Wang 2019).

With the increasing of the research year by year, molecular biotechnology has brought people into a new era of precision therapy. Targeted drugs have the advantages of safety appearance and ease of use, giving NSCLC patients a better choice. As more and more signaling pathways and drivers are discovered, drugs are increasingly diversified. Generation after generation of drugs appear, and the new generation has the tendency to surpass the old. Some drugs have their own advantages and disadvantages. How to choose the treatment strategy is the key to target therapy (Camidge et al.

2019). At the same time, drug resistance can develop after use. Drug resistance is a new challenge for targeted therapy. We need to keep the focus on new therapies to continue to make progress and treat this deadly disease. In recent decades, targeted therapy, immunotherapy and Liquid biopsy have made noticeable contributions to the improved management of lung cancer (Michela et al. 2019).

Conclusion

Patient's *BRAF* mutational status is promptly and accurately determined at the time of initial diagnosis, because it is currently the only reliable predictive biomarker that can influence the treatment of advanced lung cancer. There is high response rate and impressive survival benefit of *EGFR* tyrosine kinase inhibitors in patients harboring *EGFR* mutations. Whereas the indiscriminate use of these inhibitors can no longer be recommended. To provide patients with advanced-stage NSCLC with *ALK* inhibitors it is essential to systematically analyze for *ALK* rearrangements using a rapid, cost effective and reliable approach.*EML4-ALK* gene fusions characterize small subgroup of NSCLCs more sensitive to *ALK* inhibitors than chemotherapy and resistant to EGFR-TKIs. Though attempts to target *KRAS* pathway have shed little light so far, new molecules or new therapeutic strategies may revolutionize outcomes in patients with *KRAS*-driven NSCLC in the near future. Further investigations to better understand the pathways involved, to identify possible synthetic lethal partners and for a better patient selection are needed. A future therapeutic approach in tumors bearing p53 mutations may be to deplete cancer cells of their energy reserves and antioxidants. Personalized medicine should be based on a molecular target that drives cancer cell proliferation; the inhibition of which would cease tumor growth. As genetic and biomarker testing helpful in personalized management of lung cancer, so there is need of identifying new potential targets. With the advancement of genetic analysis, novel variations can be identified to better target treatment for individual patients.

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