Molecular Signature, Alterations, Immunohistochemical Expression and Their Impact in Lung Cancer

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Abstract:

Lung cancer is the number one cause of cancer-related death in the western world. Its incidence is highly correlated with cigarette smoking, and about 10% of long-term smokers will eventually be diagnosed with lung cancer, underscoring the need for strengthened anti-tobacco policies. Among the 10% of patients who develop lung cancer without a smoking history, the environmental or inherited causes of lung cancer are usually unclear. There is no validated screening method for lung cancer even in high-risk populations and the overall five-year survival has not changed significantly in the last 20 years. However, major progress has been made in the understanding of the disease and we are beginning to see this knowledge translated into the clinic.

Keywords: Molecular Basis, Immunohistochemical Expression, Lung Cancer.

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Introduction:

Different spectra of molecular alterations have been associated with all histological subtypes of lung cancer. Several somatic mutations and chromosomal aberrations that contribute to lung tumorigenesis (1).

the American Society of Clinical Oncology (ASCO) has recommended routine mutation testing for driver genes including EGFR, ALK, ROS1 and BRAF in clinical practice for patients with metastatic NSCLC. Although there are currently no targeted drugs for Kirsten rat sarcoma (K-RAS) or neuroblastoma rat sarcoma (N-RAS) mutated NSCLCs (2).

Main druggable genetic alterations in non-small cell lung cancer, namely EGFR, KRAS, BRAF, MET, and HER2 mutations or amplification, as well as ALK and ROS1 fusions. All have predictive impact on the outcomes of modern targeted therapies, global prognostic significance, and mutual interaction in cases of co-occurrence (3).

Importance of oncogenic driver mutation studying :

Lung cancer occurs in a multistep model and is believed to arise from genome-wide tumorigenesis events, The understanding of this model is highly beneficial in deciphering the appropriate treatment of Lung cancer and its precursors based on etiopathogenesis **(4)**

Driver mutations have significantly altered the diagnostic work-up and reshaped the oncology treatment paradigm. Recently, several new driver mutations have been identified in metastatic NSCLC, with some leading to therapeutic success and others, failure **(5)**

The identification of such mutations through genetic testing is now used for clinical diagnosis, complemented with standard hematoxylin- eosin staining of the cancerous tissue and the immunohistochemical detection of ADC or SqCC markers **(6)**

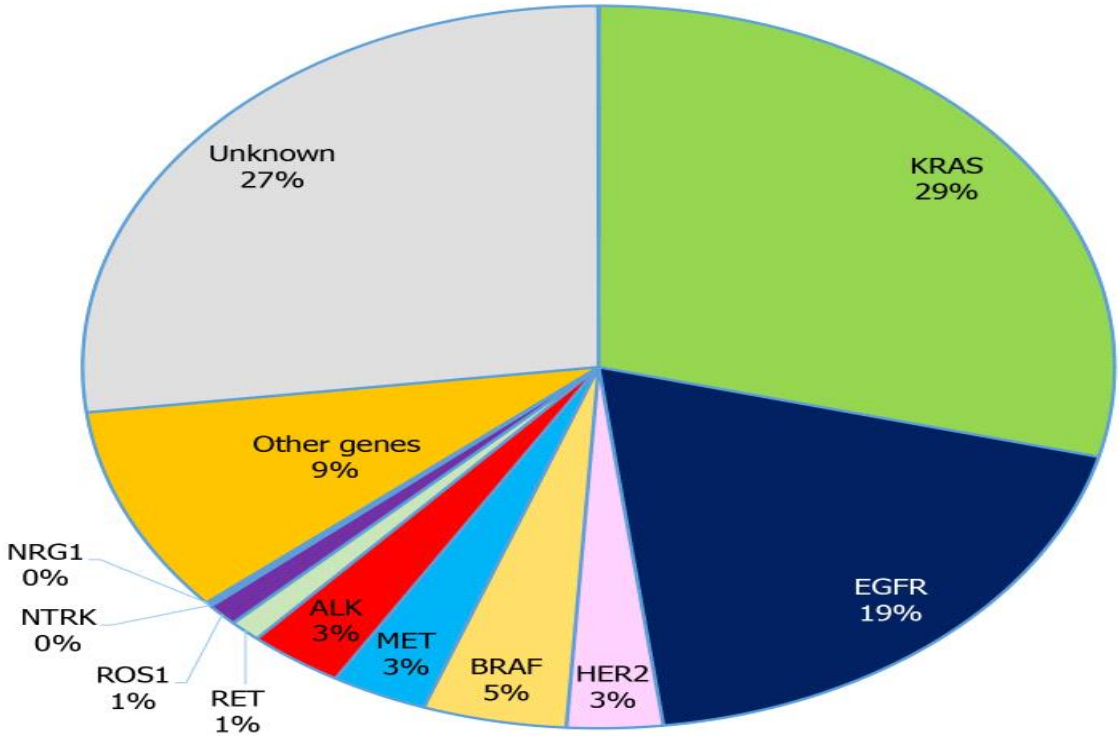

Figure 1: Incidence of oncogenic drivers in non-small cell lung cancer. KRAS: Kirsten rat sarcoma; EGFR: Epidermal growth factor receptor; ALK: Anaplastic lymphoma kinase; HER2: Human epidermal growth factor 2; ROS1: c-ROS oncogene 1; NTRK: Neurotrophic receptor tyrosine kinase; RET: Rearranged during transfection; NRG1: Neuregulin-1. (5)

1-K-RAS <u>The rat sarcoma (RAS) genes (KRAS, NRAS, Harvey rat sarcoma viral oncogene homolog):</u>

Represent the most frequent human oncogenes. Up to 30% of NSCLC harbor a mutation in the KRAS oncogene, making KRAS the most commonly detected oncogenic driver in lung cancer **(2)**

The KRAS proteins belong to the small guanosine triphosphatese (GTP) family, involved in intracellular signaling , KRAS mutations, such as those in exons 2 and 3, which prevent GTP hydrolysis and prevent switching KRAS signaling off, result in a constitutive activation of KRAS proteins. KRAS mutation is more frequent in adenocarcinoma, and can be detected by next generation sequencing (NGS) **(7)**

Approximately 13% of NSCLC have The KRAS G12C mutation which is one of most common two mutations of KRAS gene involve a substitution in the codon 12 or 13 **(8)**

2-EGFR, (HER 1) : Epidermal growth factor receptor

This protein is a member of a family of four structurally related tyrosine kinase receptors (RTKs) that includes human epidermal growth factor receptor 2 (ERBBB2/HER2), ERBB3/HER3, and ERBB4/HER4. EGFR, upon activation by specific ligands, triggers the receptor's intrinsic tyrosine kinase activity **(9)**

The receptor activation triggers a complex downstream signaling network which leads cell replication through transduction cascades which include the mitogen-activated protein kinase/extracellular signal-related kinase (MAPK/ERK) pathway, the PI3K-AKT-mTOR pathway, the JNK pathway, and others implicated in cell migration, adhesion, and proliferation pathways **(10).**

Exon 19 deletions or L858R point mutations in exon 21 account for 90% percent of the activating mutations in the tyrosine kinase domain of EGFR, resulting in constitutional activation of EGFR without ligand-induced stimulation, thus promoting cell proliferation, survival, and dissemination **(11).**

EGFR mutations are almost exclusively observed in NSCLC patients with adenocarcinoma, rather than in those with other histologies; this observation is even more striking in East Asian populations, where EGFR mutations are present in up to 78% of adenocarcinomas, as opposed to only 10–16% of adenocarcinomas in other ethnicities **(12).**

The prevalence of EGFR mutation varies with histotype, ethnicity, and other demographic or pathological factors. Several studies have shown that sensitizing EGFR mutations are most common among non-smokers, young, female & Asian lung cancer patients **(13).**

3-MET : Hepatocyte growth factor receptor

The proto-oncogene MET is located on chromosome 7q21-q31. It encodes for a transmembrane receptor (c-Met or MET) . This tyrosine kinase receptor activates downstream RAS/ERK/MAPK, PI3K/AKT, Wnt/β-catenin, and signal transducer and activator of transcription (STAT) signaling pathways, that can drive cell proliferation, survival, migration, invasion, angiogenesis, and transition from epithelial to mesenchymal **(14)**

Dysregulation of MET signaling has been found in a variety of cancers through different mechanisms, such as activating point mutations of the MET gene, over expression of the ligand HGF, MET gene copy number gain (MET-CNG)/amplification, and MET gene fusions **(15)**

MET amplification occurs in 1–6% of NSCLC cases and was considered as a negative prognostic

factor as MET amplification-mediated acquired resistance to TKIs developed from MET copy number gains and amplifications (**16)**

MET, together with EGFR, ALK, BRAF, etc. are all members of this family, which were found to be frequently mutated in advanced NSCLC **(17).** The underlying mechanism by which MET amplification leads to EGFR-TKI resistance may be associated with phosphorylation of ErbB3 (HER3), which functions as a key activator of the PI3K/AKT and MEK/MAPK pathways, providing bypass signaling in the presence of EGFR-TKIs (**18)**

4-BRAF: V-Raf murine sarcoma viral oncogene homolog B

(BRAF) gene encodes BRAF kinase, a member of mammalian cytosolic serine/threonine kinases, which plays important roles in cell signaling, growth, and survival **(14).** BRAF-mutated NSCLC patients are commonly males and smokers

A small fraction (2–4%) of NSCLC harbors BRAF mutations. BRAF-mutated NSCLC patients are commonly males and smokers . A BRAF V600E-mutation specific antibody has been developed and proven useful in detecting BRAF V600E mutations in colon cancer, but hardly detects any of the proteins encoded by non-V600E mutations **(19).**

In NSCLC 40–50% of the BRAF mutations are non-V600E, thus BRAF V600E-mutation specific IHC does not appear to be useful **(20).**

Next-Generation Sequencing (NGS)

Simultaneous targeted DNA- and RNA-based next-generation sequencing (NGS) offers the most straightforward and comprehensive profiling for all treatment relevant genetic alterations, including the genetic aberrations, which cannot be identified by FISH but are of high clinical significance; therefore, NGS has also been widely applied in clinical practice for detection of MET copy number gains and other resistances **(21)**

Two methods are commonly used for NGS-targeted approaches: capture hybridization-based sequencing and amplicon-based sequencing, and each has its own advantages and disadvantages. A head-to-head study compared these two types of methods, and indicated that amplicon-based approaches have a much-simplified workflow, and require smaller amounts of DNA for assessment. By contrast, hybridization-based NGS profiling was less likely to miss mutations, and performed better with respect to sequencing complexity and uniformity of coverage **(17).**

Immunohistochemistry (IHC) as an indicator to genetic mutation level

Mutated genes overexpressed in cancers that harbor an activating genomic signature giving an idea about oncogenic driver mutations **(17).**

The College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) issued updated guidelines for LC testing in 2018. These guidelines recommend routine multigene testing of all advanced NSCLC with an adenocarcinoma component for EGFR mutations and ALK and ROS1 rearrangements, together with additional genes (RET, MET, Her2, KRAS, and BRAF) to be more accurate **(22)**

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