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Lung Cancer Heterogeneity and New Strategies for Drug Therapy

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lung cancer, heterogeneity, therapeutic strategy, gene mutations, target drugs

Abstract

Lung cancer heterogeneity plays an important role in the development of drug resistance. Comprehensive molecular characterizations of lung cancer can describe hereditary and somatic gene changes, mutation, and heterogeneity. We discuss heterogeneity specificity, characterization, and roles of PIK3CD, TP53, and KRAS, as well as target-driven therapies and strategies applied in clinical trials based on a proposed precise self-validation system. The system is a specifically selected strategy of treatment for patients with cancer gene mutations and heterogeneity based on gene sequencing, following validation of the strategies in the patient's own cancer cells or in patient-derived xenografts using their own cancer cells isolated during surgery or biopsies. These results will be more precise if the drugs used in the strategies are selected through protein structure–guided compound screening or a DNA-encoded chemical library before validation in the patient's own cancer cells. Thus, a deeper understanding of heterogeneity mechanisms and improved validation of the therapeutic strategy will result in more precise treatments for patients.

INTRODUCTION

Precise self-validation system: a system to screen and optimize therapeutic strategies for targeted-drug efficacy and specificity using the patient's own cancer cells for that individual's treatment

Precision medicine aims to identify and develop highly selective drugs and therapies directed at disease-specific targets as an approach to drug discovery and development (1). One suggested strategy for precision medicine is to discover drugs for patients with targetable alterations of gene fusions (crizotinib for ALK fusion proteins), mutations (vemurafenib for the BRAF *V600E* mutation), methylation and acetylation, aberrations and variants, or protein overexpression (trastuzumab for HER2 proteins). Another strategy is to combine drugs based on gene sequencing, gene interactions and networks, or functional mechanisms. The efficacy, toxicity, and selection of drug combinations are highly dependent on tumor heterogeneity, as per the principle of combination design (2). The effects of therapeutic strategies are associated with the heterogeneity between intra- and intertumors, primary tumor and metastasis, tumor cells and circulating tumor cells, and inter-single cells. The forms and specificities of gene mutations may identify optimal therapies in precision medicine (3).

Lung cancer is the leading cause of cancer-related death and has a high incidence coupled with a 5-year survival rate of less than 17% (4). Small-cell lung carcinomas (SCLCs) account for 20% of lung cancers, whereas the remaining 80% are non-small-cell lung carcinomas (NSCLCs) that include adenocarcinoma (AD), squamous cell carcinoma (SCC), and large-cell carcinoma. We initially described five critical elements to ensure appropriate clinical application of precision medicine (5). We propose using tumor heterogeneity to understand the association between gene mutations and patient phenotypes, precise measurements, specific biomarkers, target-based drugs, and regulation of clinical performance. Systems heterogeneity demonstrates the full picture of heterogeneity with multidimensional functions by integrating gene or protein expression, epigenetics, sequencing, phosphorylation, transcription, pathways, and interactions (6). From a mutation perspective, targeting one mutation may be an alternative treatment strategy for all tumors with that mutation (see the sidebar titled A New Strategy: Precise Self-Validation System). The present review illustrates molecular evidence and mechanisms of heterogeneity and heterogeneity-based therapeutic strategies with a focus on lung cancer and other cancers that are similar to lung cancer. We discuss roles of hereditary and somatic gene changes when considering therapeutic strategies from multiple angles as well as genomic instability in potential mechanisms of gene mutations and heterogeneity. We also address influences of the targeted molecule subunit phosphatidylinositol 3-kinase catalytic subunit alpha (*PIK3CD*), the tumor suppressor gene *TP53*, and the carcinogenic driver *KRAS* in determining the therapeutic design. We introduce a new therapeutic strategy of a precise self-validation system using the patient's own cells to screen proposed strategies to tailor them to the individual.

HEREDITARY GENE CHANGES AND HETEROGENEITY

Genetic heterogeneity, as an influential factor in drug therapy, is highly dependent on hereditary gene changes and components in many cancers. Screening for lung cancer susceptibility

A NEW STRATEGY: PRECISE SELF-VALIDATION SYSTEM

Therapies for lung cancer are entering a new era of therapeutic strategies with a complete package of solutions to treat cancer cells based on gene changes, mutations, and heterogeneity. This precise self-validation system integrates the identification of target gene mutations and heterogeneity with nonspecific therapy, the first-line strategy of target therapy, strategies against drug resistance, the proposed strategy without validation, self-cancer cell validation, and PDX after protein structure–guided or DNA-encoded chemical library–based drug screening.

loci has demonstrated that heterogeneity was reduced in patients with similar clinical characteristics (e.g., age at onset and pattern of inheritance), based on the homogeneity and heterogeneity scores (7). A study of over 30,000 patients with lung cancer emphasized that hereditary gene changes play important roles in lung cancer histological subtypes and histology-specific germ-line susceptibility to lung cancer risks (8). The integration of 515,922 genotyped single-nucleotide polymorphisms with histopathology showed that the activity of telomerase reverse transcriptases on chromosome 5p15.33 could increase the risk of AD. A comprehensive molecular characterization of cancer will benefit the identification of subtype-specific biomarkers for early diagnosis and therapy. Researchers detected numerous hereditary gene changes in patients with chronic obstructive pulmonary disease with a high risk of developing lung cancer. They are considered as specific biomarkers to monitor the transition from chronic lung diseases to cancer (9–11).

Heterogeneity in germ cells can be amplified by the cellular offspring. Germline mutations are used to develop single or combination therapy and to discover new strategies for early diagnosis and precision prevention against cancer (12). With the development of biotechnology, the comprehensive molecular characterization of cancers associated with hereditary predispositions can be reevaluated and re-categorized for the precise assessment of disease risk and development of novel interventions. Numerous cancer susceptibility genes (e.g., *BRCA1* and *BRCA2*, *APC*, and *TP53*) are valuable for clinical diagnosis and therapy, and researchers have identified their germline mutations in family-based patients with lung cancer. Conversely, gene mutations can be cancer-causing genetic changes and can be developed or acquired as somatic changes. Hereditary gene changes and heterogeneity are more sensitive to challenges and susceptible to cancer after a few exposures to carcinogenic substances, whereas somatic changes and heterogeneity occur after more frequent and serious exposures (**Figure 1*a***).

Comprehensive molecular characterizations, or profiles of cancer, are critical in the exploration of hereditary and somatic gene changes in cancer to pierce the armor of lung cancer (13). For example, a comprehensive molecular characterization of phaeochromocytomas and paragangliomas demonstrated pathogenic germline mutations of eight susceptibility genes, including *CSDE1* as a somatically mutated driver gene; *HRAS*, *RET*, *EPAS1*, and *NF1* as four known complementary drivers; and *MAML3*, *BRAF*, *NGFR*, and *NF1* as disease fusion genes (14). Such comprehensive molecular characterizations of hereditary gene changes and heterogeneity have led to a new level of evidence-based practice of genome medicine in lung cancer. Alterations of hereditary gene mutations are measured by pedigrees, germline mutations, missenses, and mismatch repair deficiencies in lung cancer (**Figure 1*b***). The comprehensive molecular characterizations of hereditary breast cancers revealed 42 deleterious germline mutations in 21 genes of 34 patients, including 18% in *BRCA1* or *BRCA2*, 3% in *TP53*, 5% DNA mismatch repairs, 1% in *CDH1*, 6% in the Fanconi anemia pathway, and 9% in others (15). The more alterations of *TP53* germline missense they had, the easier it was to detect the risks of early-onset colorectal cancer and clinical phenotypes of patients with Li-Fraumeni syndrome (16). The patients with lung cancer also had those hereditary gene mutations, among which the histopathological subtypes clearly varied (17–19). Currently, there is a lack of comparisons to define the existence of hereditary heterogeneity and its association with mutations. One challenge is to identify the hereditary gene mutation and heterogeneity in patients with cancer, as most real-life cases have no access to family members and pedigrees. The majority of hereditary genes may also be somatic, as they are measured in patients from only one generation.

SOMATIC GENE CHANGES AND HETEROGENEITY

Somatic gene changes and heterogeneity are other important factors that alter the efficacy of drug therapy. Clinical studies on the comprehensive molecular characterization of somatic gene

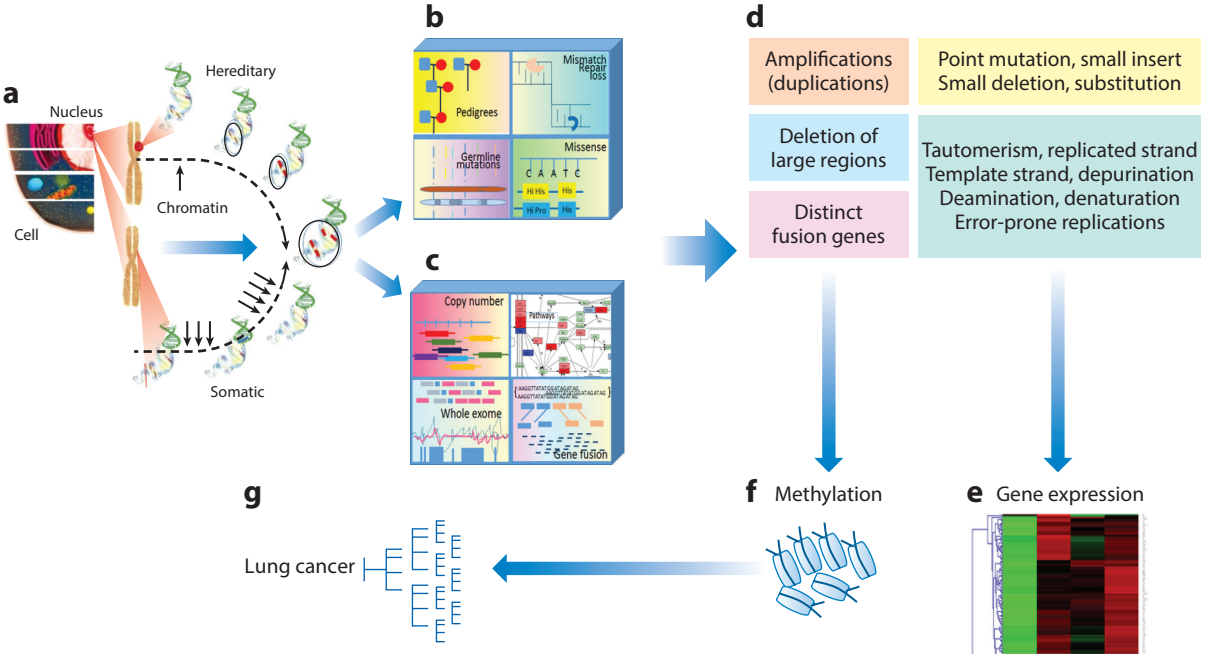

Figure 1

Molecular categorization of lung cancer originating from hereditary and somatic heterogeneity. (*a*) Hereditary gene mutation occurs after a challenge, whereas somatic gene mutation requires multiple and repeated challenges. (*b*) Hereditary alterations of germline gene mutations can be cancer risks and can influence sensitivities to therapies. (*c*) Somatic alterations of molecular differences exist between disease subtypes. (*d*) Gene mutation types, forms, numbers, epigenetics, sequencing, or heterogeneity are associated with specific subtypes of disease. Molecular categorization of lung cancer (*g*) can be defined according to the gene expression profiles (*e*) and methylation levels (*f*).

mutations in cancer are increasing in number and introduce new ways to understand the pathogenesis of lung cancer. George et al. (20) evaluated comprehensive molecular characterizations of somatic genome alterations in SCLC using mutation rates followed by a correction for expressed genes, regional clustering of mutations, genes with likely damaging mutations, biological relevance established in SCLC mouse models, and genes with likely therapeutic relevance. They found that the biallelic inactivation of *TP53* and *RB1* was altered in more than 90% and 65% of SCLC and 100% and 93% of SCLC without chromothripsis, respectively. The gene inactivation included mutations, translocations, homozygous deletions, hemizygous losses, and copy-neutral losses of heterozygosity and losses at higher ploidy. Linehan et al. (21) applied comprehensive molecular characterizations to understand and define the molecular categories of papillary renal cell carcinoma through analysis of whole-exome sequencing, copy number, mRNA, microRNA, methylation, and proteomics. Those two clinical studies are considered a new milestone and are practical examples of the use of molecular oncology to define somatic alterations at the molecular level and to identify the specificities between disease subtypes (**Figure 1*c***). It is important to screen for all types of somatic gene mutations (**Figure 1*d***) and to integrate the data with clinical phenotypes, responses to therapy, and histopathology.

Several methylation-, gene expression–, mutation-, and heterogeneity-based categories of cancer have been generated from the integration of gene mutation types, forms, numbers, epigenetics, sequencing, heterogeneity, and specificity with clinical informatics (22–24). Mutations of *MET*,

a proto-oncogene, receptor tyrosine kinase, were selected as papillary renal cell carcinoma type-specific alterations, of which 18% were germline and 90% were in the tyrosine kinase domain (21). However, MET and its ligand hepatocyte growth factor play an important role in the development of multiple cancers (25). For example, MET-dominated signals contributed to the metastasis of cancer cells to the lung by stimulating the hyperproduction and overactivation of cytokines and enzymes. Heterogeneity of MET was found to drive the resistance and treatment failure of MET inhibition in patients with MET-amplified esophagogastric cancer (26). The heterogeneity in MET amplification between distinct metastatic lesions and primary tumors led to the failure of MET inhibition despite the origination of those tumor cells from the same clonal source or their lack of any MET amplification. Although a comprehensive molecular characterization contributes greatly to our understanding of the roles of hereditary and somatic gene changes in the evolution and development of cancer, it would be even more important to explore the effect of hereditary and somatic gene heterogeneity in cell resistance and responses to drug therapy and to define the specificities of hereditary and somatic genes corresponding to disease durations, stages, severities, molecular subgroups, clinical phenotypes, responses to therapy, and prognoses of patients. Hereditary and somatic gene changes will provide new clues to understanding pathogeneses, developing therapy, and improving prevention, as well as improving our understanding of their role in the development of drug resistance, reoccurrence, and metastases in cancer.

GENOMIC INSTABILITY IN LUNG CANCER

One of the important mechanisms by which hereditary and somatic heterogeneity occurs is the formation of genome instability, which is defined as higher than normal rates of mutation and has catastrophic consequences for the development of cancer. Many pathways contribute to and promote genomic instability, including telomere damage, centrosome amplification, epigenetic modifications, and DNA damage from endogenous and exogenous sources. Tubbs & Nussenzweig (27) recently presented a comprehensive review on the knowledge and understanding of genomic instability, and they emphasized the importance of endogenous sources of mutation and epigenomic features in the balance of genomic stability and instability during cancer evolution. DNA repair pathways control and regulate the process of converting single-stranded DNA breaks to double-stranded DNA breaks through mechanisms of homologous recombination and nonhomologous end joining. De Bruin et al. (28) initially demonstrated lung cancer evolution defined by spatial and temporal diversity in genomic instability processes, including cancer evolution, intratumor heterogeneity of nonsilent mutations, the extent of genomic diversity, regional heterogeneity of potential driver mutations, and dynamics of the mutational processes. This particular study identified 1,884 nonsilent and 76,129 silent mutations during the evolution of lung cancer, among which the spatial intratumor heterogeneity in NSCLCs was composed of 26% heterogeneous mutations and consisted of 74% ubiquitous mutations in AD. One of the most important findings was that apolipoprotein B mRNA editing enzyme catalytic–associated mutagenesis, as an additional genomic instability process, might contribute to tumor progression. This phenomenon was observed as enzyme-associated mutations in tumors that consistently increased over time, as evidenced by pronounced intratumoral heterogeneity in copy-number alterations, translocations, and mutations associated with enzyme cytidine deaminase activity, in contrast to smoking-related mutations (28).

New therapeutic strategies based on genomic instability could include the prevention of DNA damage, enhancement of DNA repair, targeting of deficient DNA repair, impairment of centrosome clustering, or inhibition of telomerase activity (29). It seems that a target gene can dominate and direct the progression of genomic instability and be selected as a therapeutic pathway. For

example, $Kras^{LA2}$ was found to be a key player in tumor progression by repeating DNA copy alterations in certain genetic conditions (30). The progression of genomic instability was correlated with an increased tumor size. Ferguson et al. (29) suggested several therapeutic candidates against genomic instability (e.g., vitamins D and B, selenium, carotenoids, poly(ADP-ribose) polymerase inhibitors, resveratrol, and isothiocyanates) that may have direct or indirect effects on the maintenance of genomic stability. The great challenge is to identify hereditary or somatic gene changes and heterogeneity as well as DNA repair deficiencies in lung cancer, especially among different subtypes, stages, and severities. Measurements of gene sequencing and epigenetics are still in the process of being improved. Numerous genes have been identified as driver genes, in which a deletion could shift cancer cells back to normal cells or reduce cancer cell malignancy in preclinical studies. However, it remains poorly understood how the dysfunction of DNA repair mutation genes contributes to cancer initiation, progression, organ and tissue specificity, and metastasis in patients. With improvements in technology, gene editing may have potential for gene correction and programming repair and has been proposed as the future therapy for cancer (30–33). Komor et al. (33) recently developed a new method to directly and irreversibly convert the target DNA base into another without changing the double-stranded DNA breaks or donor template [e.g., from cytidine to uridine, resulting in a C→T (or G→A) substitution]. Gene editing has been listed as one of the hopes in clinical and translational medicine in the Cancer Moonshot 2020 program (34).

KEY GENE HETEROGENEITY

Many gene heterogeneities participate in and influence drug efficacy and resistance. Of those, we address the roles of three different categories, including a targeted molecular subunit of the key signal regulatory gene, *PIK3CD*; a tumor suppressor gene, *TP53*; and a carcinogenic driver, *KRAS*, in decisions concerning therapeutic design. PIK3CA, together with a p110 catalytic subunit, phosphorylates the 3′-position of the inositol ring and contributes to the production of phosphatidylinositol-3-phosphate, phosphatidylinositol-3,4-bisphosphate, and phosphatidylinositol-3,4,5-trisphosphate. PIK3CD has a C-terminal kinase catalytic, helical, Ras-binding, adaptor-binding, or N-terminal p85 binding-like domain (35). PIK3CD is one of the lung cancer–stem cell property-associated signaling pathways that contribute to carcinogenic potential–associated molecular mechanisms (36). The heterogeneity of PIK3CD between subtypes of lung cancers and among cancers has an important impact on the development of new therapeutic strategies (37). PIK3CA is crucial for promoting cell division through binding of pleckstrin homology domain–carrying signaling protein. Lung carcinogenic processes may be initiated whenever gene changes and mutations in PIK3CD, PIK3R1, or other PIK3 subunits occur and then fail to control the signaling and production of PIK3 phosphorylation.

PIK3CA heterogeneity among lung cancers is associated with the sensitivity and resistance of cancer cells to drugs. Lung SCC originates from basal cells and AD from alveolar epithelial cells, but SCC has a poor survival rate in comparison to AD (38) because mutations occur more frequently in lung SCC (∼9%) compared with AD (∼3%) (39). *PIK3CA* mutations, together with mutations in other genes such as *TP53*, *LKB1*, and *p63*, have been observed in all stages of NSCLC, and the number of mutations was correlated with the stage, severity, progression, and prognosis of lung cancer (40). Moreover, the interaction of *TP53* as a comutation with *PIK3CA* and *H1047R* resulted in an accelerated onset of lung cancer. The association of *PIK3CA* with *p53* mutations and expression may be responsible for signaling pathways in lung cancer. The production of phosphoinositide 3,4,5 trisphosphate, regulated by PIK3CD, plays a crucial role in human oncogenesis, during which gene changes and mutations in PIK3CD can occur through the interaction with PIK3R1. PIK3R1 has an inter-Src homology 2 domain located between the nSH2 and cSH2

domains, a GTPase-activating protein domain, and finally an Src homology 3 domain. For example, the *E334K* and *E525K* mutations of *PIK3CD* are generated through the interaction of PIK3R1 nSH2 with PIK3CD C2 and E1021K by PIK3R1 nSH2 and cSH2 with C-lobe in the kinase domain of PIK3CD. Multiple mutations in both *PIK3CD* and *PIK3R1* act as key players responsible for the pathogeneses of diseases and may represent potential new therapeutic strategies (41).

Numerous inhibitors of PI3K pathways act through different mechanisms; for example, wortmannin is an irreversible inhibitor that exerts its effect by binding covalently to a lysine residue that affects phosphate binding in cells (42), and LY294002 is a reversible and ATP-competitive PIK3 modulator (43). There are many obstacles to the clinical application of PIK3 inhibitors owing to poor specificity, solubility, stability, and pharmacological properties (44–46). Another challenge is the achievement of a high specificity of PIK3 inhibitors against not only the targeting molecule but also the different types of organ-specific cancers. For example, everolimus and LY294002, an mTOR inhibitor and a PIK3 inhibitor respectively, have inhibitory effects in cancer. Mutations become the predominant factor regulating cancer cell proliferation, with unique and specific effects that should be precisely identified and targeted. It will also be important to explore how mutant *PIK3CA* promotes the proliferation and metastasis of lung cancer cells and how it differs from other cancers. The specificity of drugs to different domains of *PIK3CD* is highly anticipated and is considered one of the greatest challenges in drug discovery and development (37).

Tumor suppressor genes (e.g., *TP53*, *BRAC1/BRAC2*, *PTEN*, *RB1*, and *APC*) play important roles in DNA damage and repair, mutations, and carcinogenesis, among which the methylation profiles are correlated with the clinical prognoses of patients with lung cancer (47). Many genes in single or multiple signaling pathways function as tumor suppressors (e.g., FBXW2 dominates the β-TrCP-FBXW2-SKP2 axis as a tumor suppressor in lung cancer) (48). There is great potential for tumor suppressor gene–targeted drug discovery and development in lung cancer, although many challenges remain, such as the targeting accuracy and stability, genotoxicity, and biological biomarkers. *TP53* mutations are considered a unique feature of cancers and exhibit a high prevalence and sensitivity, although *TP53* mutations with an extremely low frequency have also been detected in normal tissue (49). *TP53* gene changes can shift cell phenotypes toward cancerous characteristics, increase genomic heterogeneity, and desensitize drug therapy. Patients with lung cancer and *TP53* gene changes have poor prognoses, high levels of heterogeneity, and different pathological types and clinical stage characteristics (27). Intervening with TP53 may represent a new and efficient strategy for drug therapy, even though *TP53* poses difficulties as a direct and druggable target owing to toxicity. The small hydrophobic pocket of MDM2 proto-oncogene that binds TP53 can stabilize and upregulate p53 downstream transcriptional targets (e.g., p21WAF1, BAX, and BBC3), leading to cell proliferation, which can be terminated by specific binding to that pocket. The combination of small-molecule inhibitors of the MDM2-p53 binding interaction with chemotherapy, such as cisplatin, inhibited cancer cell growth (50). More than 95% of cells sensitive to combination therapy have TP53 mutations, and combination therapy is more efficient than monotherapy. Combination therapies have led to an additive or synergistic effect in a p53-dependent manner compared with cisplatin alone, but there is no indication that the combination was more efficient than a MDM2-p53 binding inhibitor in increasing p53 activation, proliferation, and p21WAF1 protein and/or caspase-3/7 activity. Numerous TP53 regulators are undergoing clinical trials as drug candidates (e.g., APR-246, MK-1775, ALT-801, Kevetrin, SGT-53, Alisertib, AT9283, ENMD-2076, and AMG900).

KRAS acts as one of the most commonly mutated oncogenes and major oncogenic drivers in cancers, and *KRAS* heterogeneity plays an important role in the resistance of cancer cells to targeted drugs (51). *KRAS* mutation exists in approximately 30% of patients with lung AD. Mitogen-activated protein kinase signaling is critical for *KRAS*-induced lung carcinogenesis, and

MEK inhibition can prevent the growth of *KRAS*-driven lung cancers. The interaction between mutated genes of tumor suppressors (e.g., *p53* or *LKB1*) and carcinogenic drivers (e.g., *KRAS*) can modulate drug responses to target drugs and immune checkpoint inhibitors in lung cancer. MEK inhibitors alter ATP-binding proteomes of *KRAS* mutant lung cancer cells and heterogeneous drug-induced pathway signatures presented among lung cancer cell types (52). Each cell type has the specificity and heterogeneity of kinome responses and adaptive types of MEK inhibition, among which decreased mitotic kinases and increased autophagy-associated kinases are more common and homogenous. Signaling pathways have diverse coexisting KRAS mutation–oriented adaptive responses (e.g., glycolysis or gluconeogenesis). The interaction of *KRAS* with other driver genes makes adaptive ATP-binding proteome and kinome responses to therapy more diverse and varied, probably due to the comutated tumor suppressors. These findings indicate that therapeutic strategies targeting mutations in carcinogenic driver genes should include drugs to target tumor suppressor genes as a co-targeting strategy.

Patient-derived xenografts (PDX): a humanized animal cancer model in which human cancer cells are transplanted into immunodeficient animals

PATIENT-SPECIFIC IN VIVO EVALUATIONS

Researchers can transplant normal human cells into immunodeficient mice to form humanized tissues and organs (53, 54). Human cancer cells are then seeded into animals to exert pathological characteristics of human cancer cells as humanized cancer animal models (55). Ambrogio et al. (56) recently measured the combination efficacy of dasatinib with demcizumab, an anti-delta-like canonical Notch ligand 4 antibody, to target the discoidin domain receptor 1 to interfere with Notch signaling in an orthotopic model of patient-derived xenografts (PDX) with lung cancer cells derived from patients with concomitant *KRAS* mutations and *TP53* deletions. The combination therapy showed better efficacy for reducing tumor volume through increased apoptosis and necrosis, better maintenance of a long-lasting response to the combination, and increased prolongation of the reemergence of tumor growth, when compared with the standard chemotherapy protocol. This result is due to a precise targeting of the discoidin domain receptor 1/Notch1 signaling needed in tumor progression and survival. This is an example of a clinical application–driven preclinical trial for the evaluation of therapeutic strategies and provides the potential for target drug screening before clinical therapy, although the results from those three patients should be further confirmed in larger cohorts. PDX-generated paired chemosensitive and chemoresistant cancers as a model of acquired chemoresistance are developed to evaluate the combination efficacy of cisplatin and etoposide in SCLC (57). SLFN11, a factor implicated in DNA damage repair deficiency, is inhibited by the deposition of H3K27me3, a histone modification induced by EZH2 within the gene body of *SLFN11*. It would be more helpful to have a target-driven sensitive and resistant PDX model for screening before clinical application. Cancer severity–driven PDX models are categorized according to genetic and transcriptomic features of the donors for preclinical screening of drug sensitivity (58). Researchers isolated cells from lung or liver cancer tissues of patients during tumor resection and seeded them in immune-deficient mice to profile genomic characteristics and screen-selected target drugs in an auto-PDX model to guide clinical therapies (59). However, the time frame needed for model development is too long to be compatible with the urgent need for clinical decision making; the success and stability of model development must be more applicable, repeatable, and treatable; and gene heterogeneity between cells contributes to variations in model sensitivities.

PRECISE SELF-VALIDATION SYSTEM

The therapeutic strategy of precision medicine for lung cancer remains a new opportunity and challenge, even though target-based therapies have been initiated and progressed in lung cancer

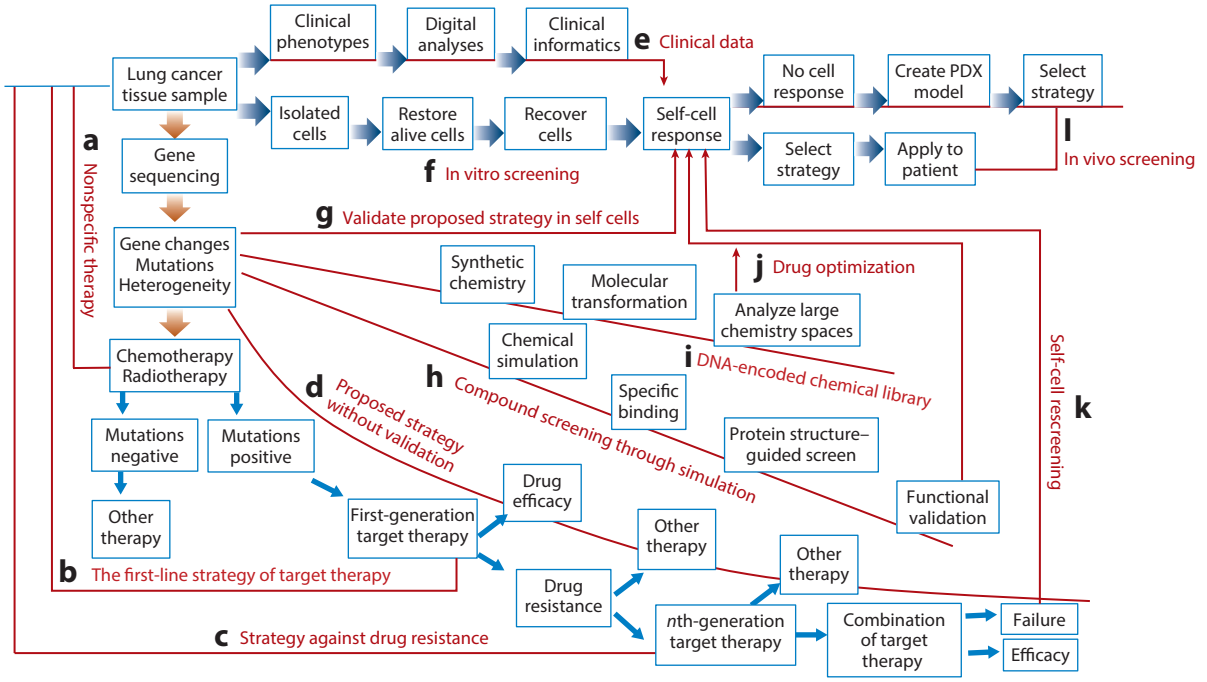

Figure 2

Content of the precise self-validation system. Patients with lung cancer usually receive nonspecific therapies such as chemotherapy and radiotherapy (*a*). Target gene inhibitors, such as the epidermal growth factor receptor inhibitor, are the first-line strategy of target therapy (*b*), and the new generation of target inhibitors are a strategy against the occurrence of drug resistance (*c*). Combination therapy would be considered in clinical practice if the new generation of monotherapy failed as the proposed strategy without validation (*d*). The precise self-validation system demonstrates that highly selected strategies integrated with clinical phenotypes and informatics against target mutations (*e*) and heterogeneity will be applied to the patient after their own cancer cells (*f*) are used to validate numerous proposed strategies based on patient gene sequencing (*g*) or in patient-derived xenografts (PDX) with their own cancer cells that have been isolated and preserved during surgery or biopsies (*l*). It will be more precise if the drugs used in the strategies can be screened and selected by protein structure–guided compound screening through simulation (*h*) or the use of a DNA-encoded chemical library (*i*) before validation in the patient's own cancer cells, to optimize hit and lead (*j*). The precise self-validation system can also be used when the proposed strategy of combination therapy fails (*k*).

[e.g., the discovery and development of epidermal growth factor receptor (EGFR) inhibition]. After chemotherapy fails (**Figure 2*a***), the first-line target therapy strategy (e.g., gefitinib, erlotinib, and afatinib) should be to measure EGFR mutations in patients with lung cancer and then treat with EGFR inhibitors if the mutation is present (**Figure 2*b***). The strategy against drug resistance is the application of new generations of target drugs following the development of resistance to the first generation of target therapy (**Figure 2*c***). For example, osimertinib as a third generation of EGFR inhibitor has better effects on *T790M* secondary mutations (60). Clinical trials have demonstrated that combination therapy has advantages toward several resistance mechanisms other than the T790M mutation. Those strategies were designed and proposed based on the target gene mutations measured by next-generation sequencing, but they were applied without further validation (**Figure 2*d***). Such strategies are highly dependent on clinical experience and individual understanding of target therapy, personalized medicine, and precision medicine.

The complexity of lung cancer (e.g., spatial and temporal tumor heterogeneity and clonal selection or evolution) makes therapeutic strategies more difficult than we anticipated. Cancer

Clinical bioinformatics: a new approach to integrate omics data with clinical phenotypes using bioinformatics tools

DNA-encoded chemical library: a technology for the synthesis and screening on an unprecedented scale of collections of small-molecule compounds

cell epigenetics, evolution, stem cells, and epithelial-mesenchymal transition play critical roles in the heterogeneity of the initiation and progression through the interaction between permanent genetic mutations and dynamic epigenetic alterations (61). The epigenetic cross talk can maintain gene transcription initiation in normal cells through a mechanism controlled by elongating RNA polymerase II with SetD2, H3K36me3, Dnmt3b, and DNA methylation (62). Subsequently, Dnmt3b-controlled methylation protects a compromised gene body caused by spurious RNA polymerase II entry and cryptic transcription initiation. In addition, intratumor microenvironments and metabolism influence gene mutations and heterogeneity during evolution and contribute to the responses of lung cancer cells to therapy (63, 64). Therefore, a new therapeutic strategy named the precise self-validation system is proposed based on clinical practice and molecular knowledge, as illustrated in **Figure 2**. Clinical phenotypes (e.g., patient symptoms, signs, biochemical measures, imaging, and responses to therapy) as the first priority are translated from descriptive information into digital data, which can be integrated with omics-based data as an important part of clinical bioinformatics (22, 23) (**Figure 2*e***). Cancer cells are isolated and harvested from lung tumors of patients with lung cancer during surgery or biopsies and are restored as alive cells. In contrast to other strategies, those alive cells can be recovered and cultured as an in vitro screening and validation system (**Figure 2*f***). Clinicians can propose numerous specific therapeutic strategies for that particular patient with lung cancer based on bioinformatics analyses of gene changes and heterogeneity measured immediately after tumor resection or biopsies. Proposed strategies of target-based single or combination therapies are then validated in the patient's own alive cells (**Figure 2*g***). This procedure will enable the treatment of patients with proposed strategies based on their own gene sequencing and own cell validating rather than strategies generated from guesses, thoughts, opinions, and experience (**Figure 2*d***). One of the practical challenges in such processes is the limited number of isolated cancer cells from human tissues, which are insufficient for use for the validation of many proposed strategies.

We recently demonstrated a new strategy for drug discovery and selection based on protein structure–guided discovery of functional mutations among cancer types to identify spatial clusters within which variants have the potentially desired function and to select target drugs for precision medication as a new potential protocol for cancer therapy (65). It is critical to understand the impact of mutations on protein structures and map variants within clusters of protein, categorize mutation-drug clusters, and prioritize clusters enriched in mutations from patient samples. The functions and efficacies of selected drug clusters from simulations of variants and drug interactions are further validated in cells with and without mutations. This philosophy is adapted for the precise self-validation system to achieve high-throughput screening of chemical binding to mutation variants through computational tools and to select optimal leads of chemical backbones, from which the category of target drugs can be indicated before screening the patient's alive cells (**Figure 2*h***). DNA-encoded chemical library technologies are used in drug discovery for hit and lead generation in the pharmaceutical process to allow the simultaneous screening of very large sets of compounds of up to billions of molecules (66, 67). One optimal approach will be to screen the lead of the chemical backbone through the library before the test in human alive cells (**Figure 2*i***). However, this technique still seems far from implementation into clinical practice due to the need for a wide variety of capabilities in aqueous synthetic chemistry, oligonucleotide conjugation, large-scale molecular biological transformations, selection methodologies, and the analysis of large chemistry spaces. Compounds identified by either protein structure–guided screening or DNA-encoded chemical libraries are validated and optimized in the patient's own alive cells to ensure patient-specific efficacy (**Figure 2*j***). Cancer cells isolated from the patient can also be applied for self-cell rescreening if the patient fails to respond to the combination therapy

(**Figure 2*k***) and for the development of patient-specific PDX models to further validate the efficacy of target drugs in the in vivo system (**Figure 2*l***).

MONITORING HETEROGENEITY

The efficacy of targeting drugs and precise strategies declines with the development of drug resistance. Heterogeneity is one of the critical factors and mechanisms responsible for drug resistance. One of the challenges in personalized clinical medicine and targeted therapies is to monitor the existence and occurrence of cancer heterogeneity, the heterogeneity-specific and associated efficacy and toxicity of targeted drugs in clinical trials, and the failure of the combination strategy, due to the lack of disease- and biology-specific biomarkers (68). The signature of *RAS/RAF* mutations predicts the sensitivity to the EGFR inhibitor cetuximab by analyzing the integration of molecular profiles with drug sensitivity patterns (58). Numerous inflammatory factors in the tumor microenvironment can be potential biomarkers to influence or reflect gene changes and heterogeneity in lung cancer (63). The variations in the immune microenvironment among lung cancer subtypes may contribute to the development of heterogeneity and drug resistance during the evolution of immune cells, cytokines, and cancer cells. The heterogeneity of epithelial cell genes during the progression of chronic lung diseases was featured as a procarcinogenic driver of the transit from chronic lung diseases into lung cancer (10). Epithelial osteopontin and mucins can act as important drivers and biomarkers in lung cancer evolution and heterogeneity (69, 70), although both may lack disease specificity and biological links with gene changes and heterogeneity as disease-specific biomarkers (71). Single-cell sequencing can be a powerful approach to detect intratumor heterogeneity and can screen mutation- and heterogeneity-specific biomarkers by integrating with single-cell imaging, biology, and system biology (72–74). Cancer stem cells, as one determining factor, contribute to intratumor heterogeneity, epigenetic modifications, and/or interactions with the tumor microenvironment (75). Droplet-based single-cell transcriptome sequencing methods are applied to define heterogeneity and accuracy. Single-cell copy-number variations reflect genomic alterations of chromosome conformation as important sources of functional heterogeneity and combinatorial cellular indexing (76).

In conclusion, the heterogeneity of lung cancer plays an important role in the development of drug resistance and the reoccurrence of the tumor. Genetic heterogeneity depends highly on hereditary gene changes and components of clinical familial characteristics, contributing to lung cancer susceptibility to drug therapy. Comprehensive molecular characterizations or profiles of lung cancer can describe hereditary and somatic gene changes, mutations, and heterogeneity by measuring mutation rates, expressed genes, regional clustering, and genes that are likely to develop damaging mutations. An imbalance between genomic stability and instability can be caused by endogenous sources of mutations and epigenomic features during the evolution of cancer. Heterogeneity specificity, characterization, and roles of PIK3CD, TP53, and KRAS have potential to uncover the mechanism and discover new therapy. The precise self-validation system first proposed in the present review can be applied for optimizing the increasing number of target-driven therapies and strategies in clinical trials to combat the large quantity and uncertainty of gene heterogeneity. Therapeutic strategies for lung cancer with detected mutations and heterogeneity usually include a nonspecific therapy, the first-line target therapy strategy, the strategy against drug resistance, and the proposed strategy with validation. The system demonstrates that highly selected strategies against target mutations and heterogeneity can be used for the particular patient after several proposed strategies based on patient gene sequencing are validated in the patient's own alive cells or in PDX with their own cancer cells isolated and preserved during surgery or biopsies. It will be more precise if the drugs within the strategies can be screened and selected by protein

structure–guided compound screening through simulation or a DNA-encoded chemical library prior to validation in the patient's own cancer cells. There is an urgent need for heterogeneity-specific biomarkers to monitor the efficacy of the strategies. Thus, the better our understanding of heterogeneity mechanisms and the better the validation of therapeutic strategies, the more precise the medication will be, leading to the best possible prognosis for the patient.

SUMMARY POINTS

1. Lung cancer is one of the leading causes of mortality and morbidity with a poor prognosis.
2. Hereditary and somatic gene changes, mutations, and heterogeneity contribute to the mechanisms by which drug resistance develops in lung cancer.
3. Therapeutic strategies vary widely owing to the heterogeneity of tumor suppressor genes, procarcinogenic genes, and known or unknown driver genes.
4. Comprehensive molecular characterizations of lung cancer subtypes provide a better chance of identifying target gene mutations and heterogeneity.
5. Genomic instability is an important mechanism of gene mutation and heterogeneity.
6. A precise self-validation system is an important approach to integrate genome medicine with clinical therapy.

FUTURE ISSUES

1. It is important to have a complete list of lung cancer gene mutation alterations and heterogeneity alterations with a clear link to their functions and sensitivities.
2. In addition to a pathological category, a new molecular category of lung cancer should be developed and applied for clinical therapies.
3. Comprehensive molecular characterizations of lung cancer should be further explored according to their severity, stage, duration, therapy, carcinogenesis, and prognosis.
4. Germline lung cancer risks should attract more attention from clinical scientists for translation into clinical practice (e.g., precision medicine and prevention).
5. The precise self-validation system must be further evaluated, validated, and promoted to improve our understanding of gene changes, mutations, and heterogeneity.
6. The precise self-validation system should be programmed and automated with a learning function and become an intelligent assistant for decision making during clinical applications.

DISCLOSURE STATEMENT

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Errata

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