Review Article

Gene aberrations for precision medicine against lung adenocarcinoma

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Lung Adenocarcinoma and Oncogene Addiction

lung adenocarcinoma is the most common histological subtype of non-small-cell lung cancer. Cigarette smoking is a major cause of lung cancer; however, among the major histological types of lung cancer, LADC is the most weakly associated with smoking, and often occurs in females and never-smokers.1,2 Lung adenocarcinoma is also the type of lung cancer in which somatic gene aberrations have been most extensively studied (Table S1).3,4 Lung adenocarcinoma can be classified according to the presence of specific mutually exclusive oncogene aberrations that drive carcinogenesis. Although many gene aberrations accumulate during the development of each individual case of LADC, these cancers are primarily driven by single oncogene aberrations that play major roles in oncogenesis and tumor progression. These aberrations are thus referred to as “driver oncogenes”. In precision medicine, tumors with driver oncogene aberrations can be treated using “molecular targeted” drugs. For example, tumors that develop due to EGFR gene mutations or ALK gene fusions respond to therapy with TKIs that suppress the kinase activities of the aberrant EGFR and ALK proteins, respectively.5,6 These therapeutic strategies are based on the concept of “oncogene addiction”, that is, the dependence of a cancer cell on a single aberrant driver oncogene for survival and growth.5

Driver Oncogene Aberrations Occur in a Mutually Exclusive Manner

The contribution of driver gene aberrations to development of LADC differs by smoking status, sex, and ethnicity. To illustrate this phenomenon, we provide pie charts in Figure 1 and Table S2 showing the distributions of driver oncogene aberrations in two cohorts: National Cancer Center Hospital, Japan (NCC_Japan cohort, consisting of 319 Japanese patients), representing Asian cases,6 and a TCGA (The Cancer Genome Atlas) study (TCGA_USA cohort, consisting of 230 US patients), representing European/US cases.7 Known driver oncogene aberrations, such as mutations of the EGFR, KRAS, BRAF, and HER2 genes, and fusions of the ALK, RET, and ROSI genes, occur mutually exclusively in both cohorts. Also, in both populations, skipping of exon 14 of the MET gene, which occurs as a result of a variety of splice site and intronic mutations,8 is observed in cases that lack the aforementioned driver aberrations. Thus, irrespective of ethnicity, a common set of oncogenes drives lung adenocarcinogenesis.
**EGFR, a Major Driver Oncogene in Asian LADC**

Despite the common features described above, the frequencies of some oncogene aberrations differ significantly between Asian and US/European populations, as is apparent in the Japanese and US cohorts (Fig. 1): EGFR mutations are more prevalent in Japanese patients, whereas KRAS and BRAF mutations are more prevalent in the US.\(^{(9)}\) KRAS and BRAF mutations occur preferentially in LADC of ever-smokers and males.\(^{(10,11)}\)

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**Fig. 1.** Frequencies of driver oncogene aberrations in lung adenocarcinoma (LADC), shown as pie charts. Frequencies are shown for mutations in **EGFR**, **KRAS**, **BRAF**, and **HER2** (driver mutations), fusions involving **ALK**, **RET**, **ROS1**, **NRG1**, and **BRAF** (driver fusions), and skipping of **MET** exon (ex) 14 (others). Data were obtained from a Japanese cohort \((n = 319)\) from the National Cancer Center Hospital, Tokyo (NCC_Japan) and a US cohort \((n = 230)\) from The Cancer Genome Atlas study (TCGA_USA).

**Fig. 2.** Frequency of driver oncogene aberrations in lung adenocarcinoma (LADC) according to smoking status (a) and sex (b). Aberrations are shown for all Japanese and US cases for which information on sex and smoking was available. The oncogene aberrations referred to in the text are emphasized by exploding pie charts. ex, exon.
Notably, Asian LADC cohorts more frequently include females and never-smokers than those of European descent. Consistent with this, EGFR mutation, which preferentially occurs in LADCs in females and never-smokers, is more frequent in Asians than in US/European individuals. Oncogene distributions are illustrated in patient populations stratified by smoking status (Fig. 2a) and sex (Fig. 2b). Frequencies of EGFR mutation were higher among never-smokers and females in both populations, but Japanese patients were more likely to have EGFR mutations than US patients in all groups stratified by smoking and/or sex (Figs 2,S1). Thus, frequent EGFR mutation is likely to be a robust feature of Japanese LADCs.

Asians, including Japanese people, might carry endogenous and/or exogenous risk factors responsible for the development of LADC with EGFR mutation. This idea is consistent with the fact that never-smoking Asians have higher lung cancer risk than never-smoking non-Asians. Notably, genome-wide association studies have shown that odds ratios for genetic polymorphisms at LADC susceptibility loci, such as those at TERT and TP63, are higher in Asians than in individuals of European descent (Table S3). In China, TERT polymorphisms are more strongly associated with risk of LADC with EGFR mutation than for LADC without such mutation. In addition, Asian-specific LADC risk loci have been discovered. Thus, genetic background (i.e., the overall complement of genetic polymorphisms) represents a potential endogenous risk factor that makes Asians more susceptible to LADC with EGFR mutation.

Oncogene Fusions Driving Lung Carcinogenesis

Like EGFR mutations, ALK, RET, and ROS1 oncogene fusions arise preferentially in LADCs of never-smokers. In fact, LADCs with these aberrations were more frequent in never-smokers than in ever-smokers in both the Japanese and US cohorts (Fig. 2a; 12.1% vs 2.5% in Japan, and 15.6% vs 2.1% in the US). Therefore, oncogene fusions are another important driver of lung adenocarcinogenesis in never-smokers.

To determine the risk factors for development of LADCs with oncogene fusions, genomic breakpoints for chromosome translocations causing ALK, RET, and ROS1 fusion were characterized by cloning and sequencing of genomic fragments containing the fusion breakpoint junctions. The breakpoints were clustered in regions of a few kilobases within the oncogenes (Fig. 3). Interestingly, the cluster region of RET included most RET fusion breakpoints observed in papillary thyroid cancers induced by the Chernobyl accident. The structures of the breakpoint junctions indicated that two DNA double-strand break repair mechanisms, non-homologous end joining (active both in replicating and non-replicating cells) and synthesis-dependent end joining (active only in replicating cells), contribute to illegitimate joining of DNA ends of oncogenes and their fusion.
partners. Thus, DNA strand breaks at specified regions of oncogenes in replicating and non-replicating lung epithelial cells are likely to be responsible for the development of LADCs with oncogene fusions, although the endogenous and exogenous factors that cause these strand breaks are unknown.

Therapy Targeting Driver Oncogene Aberrations

Targeted therapies using TKIs against tumors with EGFR mutations and ALK fusions have yielded dramatic success in precision LADC medicine. Therefore, additional driver oncogenes are being translated into molecular targeted therapies.

One representative example of a TKI target is RET oncogene fusion, discovered by our group and others. Oncogene addiction of tumors harboring this fusion and the therapeutic utility of TKIs against RET kinase activity have been demonstrated in vitro studies and in a transgenic mouse model. In addition, several LADC cases with RET fusions responded to RET TKIs approved by the FDA for the treatment of tumors other than lung cancers. Consequently, the utility of repositioning existing RET TKIs to LADC therapy is being evaluated in clinical trials, e.g., LURET (lung cancer with RET rearrangement study; clinical trial registration no. UMIN000010095) in Japan, a phase II clinical trial investigating the therapeutic effects of vandetanib. Similarly, LADC cases with ROS1 fusions have been subjected to clinical trials of ROS1 TKIs, such as crizotinib, yielding promising results. Clinical trials targeting other driver oncogene aberrations, such as BRAF and HER2 mutations, are also underway. Lung adenocarcinomas with the BRAF V600E mutation or an in-frame insertion in HER2 exon 20 respond to treatment with the BRAF inhibitor vemurafenib and anti-HER2 drugs such as trastuzumab or afatinib, respectively.

Other Infrequent Driver Oncogene Aberrations

Additional driver oncogenes have been identified that occur in a subset of LADCs lacking the oncogene aberrations described above. NF1, a tumor-suppressor gene in which mutations cause the hereditary cancer-prone disease neurofibromatosis type 1, encodes a negative regulator of RAS proteins. Recent
Distinct Driver Oncogene of Lung Tumors with Specific Subtypes

Invasive mucinous lung adenocarcinoma, a histological subtype of LADC composed predominantly of goblet cells, constitutes 5–10% of all LADC cases. Invasive mucinous lung adenocarcinoma tumors frequently harbor activating KRAS mutations. Recently, our group and others identified CD74–NRG1 fusion as another driver oncogene aberration in IMA of Japanese patients, particularly in females and never-smokers (Figs 4, S2). The NRG1 gene encodes a ligand of ERBB receptor tyrosine kinases, neuregulin/hereregulin, and its fusion to CD74 leads to extracellular expression of the epidermal growth factor-like domain of neuregulin, providing a ligand for the HER2–HER3 complex. Activated HER2–HER3 signaling increases cancer stem cell properties through an autocrine loop mediated by IGF2. Thus, IMA with CD74–NRG1 fusion can be treated using TKIs that target HER and/or IGFR kinases. Ciliated mucinous papillary tumor, a rare LADC subtype, is frequently associated with fusions of NTRK1 (TRKA) protein, which activates the MAPK, phospholipase Cγ (PLC-γ), and phosphatidylinositol 3-kinase (PI3K) pathways. Fusions of NTRK1 have been detected in oncogene-negative LADCs, although their incidence is likely to be less than 1% in the USA. Notably, the tropomyosin receptor kinase A inhibitor, entrectinib, has yielded promising results against such tumors. In addition, activating mutation of ARAF was detected in a case of LADC that was an exceptional responder to sorafenib, a multiple kinase inhibitor. Therefore, NTRK fusion and ARAF mutation represent druggable oncogene aberrations. However, no NTRK fusions or ARAF mutations have been observed in our Japanese LADC cohort, indicating that these aberrations make little or no contribution to the development of LADC in people of Japanese descent.

Precision LADC Medicine Based on Multistep Molecular Carcinogenesis

Both EGFR and KRAS mutations are detected in invasive and non-invasive tumors (adenocarcinomas in situ). By contrast, mutations in the TP53 tumor-suppressor gene are detected exclusively in invasive tumors with EGFR and KRAS mutations, but never in non-invasive tumors. Therefore, it is likely that EGFR and KRAS mutations contribute to the genesis of non-invasive tumor cells, and that TP53 aberration facilitates progression of non-invasive tumor cells to the invasive state (Fig. 5). Consistent with this, several studies reported that loss of TP53 function promotes the development of an invasive/metastatic tumor phenotype. These observations suggest that targeting cells with aberrant TP53 function, for example, by attacking vulnerabilities cause by TP53 dysfunction or restoring the function of abnormal TP53 protein, might be an effective strategy against tumors harboring such mutations.

By contrast, the majority of LADCs with ALK, RET, and ROS1 fusions are negative for TP53 aberrations as well as other cancer-related gene aberrations. At present, it is not clear whether these fusions occur in pre-invasive lung tumors; however, the fusion gene products may be able to generate LADC and promote tumor progression by themselves. Thus, these results support the current therapeutic strategy of using TKIs to suppress the activity of gene fusion products in fusion-positive LADCs.

Approximately 30% and 50% of Japanese and US LADCs, respectively, lack targetable oncogene aberrations (Fig. 1). Patients with these tumors were previously thought not to benefit from molecular targeted therapy; several therapeutic means for circumventing this situation are currently being investigated.
First, patients with driver oncogene-negative LADCs are often males and ever-smokers, and their tumors have larger numbers of mutations, especially tobacco-associated transversions, than those in never-smokers.\(^{(42)}\) Therefore, such cases should respond well to immune checkpoint blockade therapy, whose effectiveness depends on the mutation burden of tumor cells.\(^{(58)}\) Second, oncogene-negative LADCs frequently harbor deleterious aberrations in genes encoding subunits of the SWI/SNF chromatin remodeling complexes, such as SMARCA4/BRG1 and ARID1A/BAP250A (Fig. S3).\(^{(42,59)}\) Such SWI/SNF gene defects are thought to contribute to carcinogenesis through dysregulation of gene expression and cell differentiation, in cooperation with other cancer-related gene aberrations. However, these mutations also create a vulnerability in cancer cells: specifically, tumor cells with SWI/SNF defects are more dependent on functions of other chromatin remodeling genes than those without such defects. For example, SMARCA4-deficient cancer cells depend on SMARCA2/BRM, and growth of ARID1A-deficient cancer cells is dependent on EZH2.\(^{(59-62)}\) Several inhibitors for EZH2 histone H3K27 methyltransferase have been developed for the treatment of blood tumors with activating EZH2 mutations, and repositioning of these drugs represents a promising approach to treating oncogene-negative LADC.

The NKX2-1/TTF-1 gene was rediscovered as a target oncogene for focal amplification in LADC.\(^{(63)}\) NKX2-1 encodes a lineage-specific transcription factor that has an essential role in the formation of type II pneumocytes, which line the alveoli of the lung; therefore, it is a lineage-survival oncogene. Recent studies revealed that the receptor tyrosine kinase ROR1 is a transcriptional target of NKX2-1 and is a promising target for LADC therapy, irrespective of EGFR mutation status.\(^{(64,65)}\) Targeting of IGF1/2, IGF1R, and vascular endothelial growth factor receptor (VEGFR) for the therapy of LADC has also been reported.\(^{(66,67)}\) Thus, drugs targeting several oncogene products are predicted to contribute further to precision LADC medicine in the near future.

**Toward Further Improvements in Precision Medicine**

In this review, we summarized the gene aberrations that underlie carcinogenesis and guide personalized therapy against LADC. To further understand the molecular characteristics of these aberrations and improve precision medicine for LADC, we believe that studies carried out in the near future should focus on the following priorities. First, we need to identify gene aberrations that drive the development of oncogene-negative LADCs and to which cancer cells become addicted. Genetic and epigenetic aberrations that have not been detected by whole-exome sequencing studies to date may be responsible for oncogene activation and/or tumor-suppressor gene inactivation. Strong candidates for such aberrations include oncogene activation by intra-gene rearrangements, such as exon duplication in EGFR,\(^{(68)}\) or by super-enhancer formation/amplification.\(^{(69)}\) Deficiency in chromatin remodeling genes might also activate oncogenes and/or inactivate tumor-suppressor genes. Second, we must identify genes that affect the efficacy of molecular targeted therapy. Tumor response to these therapies varies even among cases harboring the same driver oncogene aberration. Elucidation of the responsible factors will enable us to improve the efficacy of existing therapies. Finally, we must identify the genetic polymorphisms or germline mutations that underlie the development of LADC interacting with the occurrence of driver oncogene aberrations. This information will aid in prevention and early, accurate detection of LADC. Because most molecular targeted therapies are given to patients with advanced cases, and eventually fail due to drug resistance, efforts toward prevention and early diagnosis will ultimately make a significant contribution to curing these diseases.

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**Disclosure Statement**

The authors have no conflict of interest.

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| ALK           | anaplastic lymphoma kinase |
| BRAF          | B-Raf proto-oncogene |
| EGFR          | epidermal growth factor receptor |
| HER           | human epidermal growth factor receptor |
| IGF           | insulin-like growth factor |
| IGFR          | insulin-like growth factor receptor |
| IMA           | invasive mucinous lung adenocarcinoma |
| LADC          | lung adenocarcinoma |
| RET           | Ret proto-oncogene |
| ROS1          | proto-oncogene |
| SWI/SNF       | switch/sucrose non-fermenting |
| TKI           | tyrosine kinase inhibitor |

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Fig. S1.** Frequency of driver oncogene aberrations in lung adenocarcinoma according to combined sex and smoking status.

**Fig. S2.** Frequency of driver oncogene aberrations in invasive mucinous lung adenocarcinoma according to sex and smoking status.

**Fig. S3.** Positions and types of SMARCA4 and ARID1A mutations in Japanese and US patients with lung adenocarcinomas.

**Table S1.** Representative large-scale genomic sequencing studies in major histological types of lung cancer.

**Table S2.** Comparison of clinical and pathological characteristics in patients with lung adenocarcinomas between a Japanese cohort from the National Cancer Center Hospital, Tokyo (NCC_Japan) and a US cohort from The Cancer Genome Atlas study (TCGA_USA).

**Table S3.** Odds ratios for single nucleotide polymorphisms in two loci associated with lung adenocarcinoma risk.

**Table S4.** Other infrequent candidate driver gene aberrations in a lung adenocarcinoma patient cohort from the National Cancer Center Hospital, Tokyo (NCC) (Saito et al., 2015).