Editorial

Development of biomarkers for real precision medicine

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Most cancers are genetically heterogeneous diseases, and many genes or gene products have altered their expressions or activities in cancer cells due to a variety of genetic and epigenetic mechanisms in DNA. Copy number aberrations (CNAs), common genomic events during carcinogenesis, are known to affect a large fraction of genome and extensively studied for last several decades. Common recurrent gains or losses of specific chromosomal regions occur at frequencies that they may be considered distinctive features of tumoral cells (1,2), whereas these alterations do not occur in normal somatic cells. Over the time, advances in high-throughput sequencing and other technologies for genomic analysis have led to new insights of numerous oncogenic processes that may be helpful for identification and development of prognostic and predictive markers. Recently, a meta-analysis of many human tumor types revealed either a large number of somatic mutations or CNAs, but usually not both in cancer (3). Numerous somatic mutations and CNAs are now being used or in the process of development for clinical use.

Recently, researchers have been prompted to develop biomarkers that can predict patient prognosis and drug-effectiveness in variety of cancers. For example, in breast cancer, the multigene signature panels, Oncotype DX and MammaPrint, predict patient prognosis by evaluating mRNA expression levels of 21 or 70 related genes respectively, and these tests are clinically utilized for the selection of patients who will and who will not receive adjuvant chemotherapy (4,5). However, despite enormous efforts and accumulated biological evidences, biomarkers development and validation are challenging, and it is difficult to implement any biomarker in clinical practice that is partially due to tumor heterogeneity and inter-laboratory differences in pre-clinical findings. When we discuss biomarkers, it is imperative to distinguish between "prognostic" and "predictive" markers. Prognostic markers reflect patient prognosis and survival, whereas predictive markers indicate whether a particular treatment brings clinical benefits to patients (6)

Lung cancer is the second most common cancer and the leading cause of cancer deaths worldwide. A hallmark of lung cancer development is the sequential genetic and epigenetic abnormalities in somatic cells (7), and a better understanding of the intrinsic biological traits that underline the initiation and progression of non-small cell lung cancer (NSCLC) may be essential for developing biomarkers to manage this disease appropriately. In NSCLC, various gene signatures have been reported as the candidates for prognostic and predictive biomarkers, but no ideal biomarker is still available for appropriate clinical use. Despite extensive efforts from the basic researchers and clinicians for last several decades, the most important prognostic factors in NSCLC are pathological stage (6). Referring to predictive markers, *EGFR* mutations (8), *ALK* gene rearrangement (9) and *KRAS* mutation (10) have been identified and validated as predictive markers, and these markers are clinically used for therapeutic decision (6). Recently, PD-L1 has been focused as a possible predictive marker for anti PD-1 blockades, and PD-L1 expression is assessed by immunohistochemistry for administrating these immune-checkpoint blockades (11,12). Among others, *ERCC1* and *RRM1* were promising in initial studies, however, the use of these molecules as predictive markers for the benefit from chemotherapy were denied in a phase 3 clinical trial (13). Although a meta-analysis reported that *BRCA1* expression had positive association with patients' prognoses treated with platinum- and toxal-based

chemotherapy (14), the following prospective study failed to prove its utility (15). In summary, these results suggested the promises and challenges of identifying prognostic and predictive biomarkers in NSCLC.

In 2018, Rotolo *et al*. reported the prognostic and predictive markers from genome-wide copy number analyses using 976 samples from NSCLC patients in *Transl Lung Cancer Res* (16). These patients were participants of Lung Adjuvant Cisplatin Evaluation project that showed the significance of postoperative cisplatin-based chemotherapy in stage I–III NSCLC (17). DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) samples, and copy number analyses were comprehensively performed. Chromosomal regions that indicated 2-fold higher or lower copy number were identified, and their associations with disease-free survival (DFS), overall survival (OS) and lung-cancer specific survival (LCSS) were evaluated.

A total of 217,611 array probes were grouped into 431 copy number regions, and 94 CNAs (51 gains and 43 losses) were detected on average. Among them, the chromosomal regions that contain *TERT*, *PIK3CA*, *MECOM* and *CCNL1* were gained most frequently, whereas the most frequent loss was observed in the region that contains *CDKN2A/B*, tumor suppressor genes that regulate cell cycle. When the associations between these CNAs and prognosis were evaluated, the loss of *CDKN2A/B* included region was associated with shorter DFS, OS and LCSS. Loss of *STK11* (*LKB1*) contained region was associated with shorter DFS. In addition, aberrations of genomic regions such as losses of *FSTL3*, *MLLT1*, *SH3GL1*, *TCF3* and *VAV1* showed poor prognosis. They further evaluated the association of CNAs with adjuvant chemotherapy response. Their analysis revealed that CNA of 14q32.33 predicted better response to adjuvant chemotherapy in both univariate and multivariate analyses and affected DFS, OS and LCSS. Regions in chromosome 10 that contain *BMI1*, *NET1*, *MAP3K8*, *MLLT10*, *ZMYND11*, *RET* and *RASSF4* showed predictive effects for the adjuvant chemotherapy that affected OS. Interestingly, this study referred to the differences of prognostic effects between adenocarcinoma (ADC) and squamous cell carcinoma (SCC). Copy number gains in the regions that contain *FCGR2B*, *PBX1*, *TPR*, *LHX4*, *CDC73* and *EGFR* were associated with shorter prognosis in ADC, whereas gains of the same regions showed longer prognosis in SCC, an opposite effect that needs to be biologically validated. Opposite clinical association for ADC and SCC was observed in other genomic region that includes *PTTG2*. These differences between ADC and SCC suggest the biological differences of these histologic subtypes. Furthermore, the authors evaluated the association between chromosomal instability and prognosis. Patients with high chromosomal instability had worse DFS and LCSS than those with low instability, whereas no association was observed between the chromosomal instability and the effect of adjuvant chemotherapy.

Although observational, this study was conducted on a large series of samples from well-designed randomized clinical trials, and copy number analysis was performed exhaustively in all autosomal chromosome regions. Because DNAs were extracted from FFPE samples, these results can be validated in different surgically resected samples, and a prospective study needs to be conducted before clinical use. Among several gene candidates for prognostic markers from this study, *CDKN2A* and *STK11* are well studied tumor suppressor genes (18,19). However, identified CNA regions that contain *FSTL3*, *MLLT1* and *SH3GL1* have not been studied in lung cancer previously. Additionally, several candidates as predictive markers of adjuvant chemotherapy reported in this study are novel and need to be confirmed biologically and clinically. Further exploration of these genes in NSCLC might provide hints for the development of new predictive tools. Because the data in this study were from copy number analysis, the association between each gene expression level and patient prognosis in the same cohort would provide more solid evidence. In addition, the validation in several publicly available data sets is warranted for further prospective study. With regard to predictive markers, sample analyses from patients who caused metastases potentially provide additional candidate genes that predict effects of drugs used in metastatic settings.

Recently, multigene signatures analyses have been adopted to anticipate patient prognosis and efficacy of a particular therapy. Combined several key gene expressions can provide more precise prospects. Oncotype DX that evaluates recurrent possibility of breast cancer by measuring the expression levels of 21 genes is one of the most successful tools, whose utility has been validated in the prospective and randomized trial (4). In NSCLC, although many multigene signature models have been developed, many of them failed to show same performance in different cohorts (6,20). At present, a few multigene prognostic signatures are commercially available, and prospective clinical trials are ongoing. One of them is myPlan Lung Cancer that assesses tumor aggressiveness in lung cancer. Thirty-one genes involved in cell cycle progression are measured simultaneously and normalized

to 15 housekeeping genes using RNA extracted from FFPE (21). Prognostic scores are created with these genes expression levels and pathological stage, and 5-year risk of lung cancer-specific mortality is calculated. This scoring was validated in the cohort of early lung cancer patients, and, according to the results, high prognostic score patients might be candidates for adjuvant chemotherapy (22). The second prognostic commercialized tool is Pervenio Lung RS platform. This assay determines the risk score from expression levels of 14 genes including three reference genes in FFPE samples from non-squamous NSCLC patients. Recent prospective and nonrandomized study that intended for stage I–II non-squamous NSCLC patients showed that 5-year DFS was 48.9% among high-scored patients without adjuvant chemotherapy, 93.8% among low-risk untreated patients, and 91.7% in high-scored patients with adjuvant chemotherapy (23). Although these assays aim to predict patient prognosis, they are potentially utilized as predictive tools for adjuvant therapy. Besides these predictive tools that use tumor materials, assessing circulating tumor DNA or cell free DNA has been attempted as less-invasive analytic tools (6,20).

In a recent few decades, basic researchers and clinicians have made huge efforts to develop prognostic and predictive biomarkers, and, now, some assays have reached to clinics and brought benefits to patients. However, these progressions are still in their infancy, and continued interdisciplinary efforts are needed for fruitful success. Further exploration possibly contributes to brushing up all the developed analytic tools. We are coming close to the real precision medicine.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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