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Original Articles

# Gene mutations in primary tumors and corresponding patient-derived xenografts derived from non-small cell lung cancer

Chuncheng Hao [a,1], Li Wang [a,1], Shaohua Peng [b], Mengru Cao [a], Hongyu Li [a], Jing Hu [a], Xiao Huang [a], Wei Liu [a], Hui Zhang [a], Shuhong Wu [a], Apar Pataer [a], John V. Heymach [b,c], Agda Karina Eterovic [d], Qingxiu Zhang [e], Kenna R. Shaw [e], Ken Chen [f], Andrew Futreal [g], Michael Wang [h], Wayne Hofstetter [a], Reza Mehran [a], David Rice [a], Jack A. Roth [a], Boris Sepesi [a], Stephen G. Swisher [a], Ara Vaporciyan [a], Garrett L. Walsh [a], Faye M. Johnson [b,c], Bingliang Fang [a,c,*]

[a] Department of Thoracic and Cardiovascular Surgery, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA
[b] Department of Thoracic and Head/Neck Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA
[c] The University of Texas Graduate School of Biomedical Sciences, Houston, Texas, USA
[d] Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA
[e] Sheikh Khalifa Bin Zayed Al Nahyan Institute for Personalized Cancer Therapy, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA
[f] Department of Bioinformatics and Computation Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA
[g] Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA
[h] Department of Lymphoma, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

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ABSTRACT

Molecular annotated patient-derived xenograft (PDX) models are useful for the preclinical investigation of anticancer drugs and individualized anticancer therapy. We established 23 PDXs from 88 surgical specimens of lung cancer patients and determined gene mutations in these PDXs and their paired primary tumors by ultradeep exome sequencing on 202 cancer-related genes. The numbers of primary tumors with deleterious mutations in *TP53, KRAS, PI3KCA, ALK, STK11,* and *EGFR* were 43.5%, 21.7%, 17.4%, 17.4%, 13.0%, and 8.7%, respectively. Other genes with deleterious mutations in ≥3 (13.0%) primary tumors were *MLL3, SETD2, ATM, ARID1A, CRIPAK, HGF, BAI3, EP300, KDR, PDGRRA* and *RUNX1*. Of 315 mutations detected in the primary tumors, 293 (93%) were also detected in their corresponding PDXs, indicating that PDXs have the capacity to recapitulate the mutations in primary tumors. Nevertheless, a substantial number of mutations had higher allele frequencies in the PDXs than in the primary tumors, or were not detectable in the primary tumor, suggesting the possibility of tumor cell enrichment in PDXs or heterogeneity in the primary tumors. The molecularly annotated PDXs generated from this study could be useful for future translational studies.



## Introduction

Lung cancer is the leading cause of cancer-related deaths both in the United States and worldwide, with an annual global incidence of about 1.6 million and mortality of 1.4–1.5 million [1–3]. Recent advances in genomic profiling have led to the identification of a number of frequently mutated genes in lung cancer [4–7]. Lung cancers with the same histological diagnosis and clinical stages can be classified into molecular subgroups based on gene mutations. Substantial efforts have been made to develop genotype-specific anticancer therapeutics. The finding that lung cancer cells with mutations in the epidermal growth factor receptor gene (*EGFR*) are highly susceptible to the EGFR inhibitors gefitinib [8–10], erlotinib [8,11] and afatinib has made these agents the first choice for treating *EGFR* mutant lung cancer. Both gefitinib and erlotinib have been reported to significantly prolong progression-free survival in patients with *EGFR*-mutant lung cancer [12,13]. Similarly, small molecular inhibitors for anaplastic lymphoma kinase (ALK) and ROS1 have been proven to be highly effective for treatment of lung cancers with *ALK* and *ROS1* gene translocations [14–16]. However, despite the excitement accompanying the targeted therapeutics, only a subset of patients with the aberration respond and responses are often unfortunately brief. Furthermore, our knowledge of genetic alterations, their functional consequences and combinatorial effects in lung

* Corresponding author. Tel.: +1 713 563 9147; fax: +1 713 794 4901.
*E-mail address:* bfang@mdanderson.org (B. Fang).
[1] These authors contributed equally.

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cancer is still not comprehensive. For most potential driver mutations identified in lung cancer, there are no effective therapeutic agents available. The success of the EGFR inhibitors underscores the urgency of developing effective genotype-specific anticancer therapeutics.

Anticancer drug development is often impeded by a lack of preclinical tumor models that are highly predictive of therapeutic effects in humans. Previous studies have shown that *in vitro* cell line models and *in vivo* xenograft tumors derived from established human cancer cell lines have limited predictive value for antitumor activity of a drug in clinical trials [17–19]. Anticancer agents that showed promising *in vivo* antitumor activity in xenograft tumor models have often been ineffective for the same type of cancer in clinical trials [20]. In fact, only about 5% of anticancer agents evaluated in human studies between 1991 and 2000 were successfully registered [20]. The majority of failures in late-phase clinical trials result from a lack of clinical efficacy caused primarily by the lack of efficacy proof of concept in humans, lack of predictive biomarkers to identify patient responders, and safety issues [20,21]. Thus, clinically relevant tumor models that accurately predict therapeutic efficacies would be highly valuable for anticancer drug development.

Evidence from recent studies has shown that patient-derived xenografts (PDXs) established directly from patients' primary tumors preserve the histomorphologic features, heterogeneity, gene expression pattern (including cytokine expression by tumor stromal cells), DNA copy number alterations, and gene mutations of the original tumors [22–24]. These features were preserved after a series of passages of the tumorgrafts in mice [22,24]. When PDXs were treated with agents used in a parallel patient population, response rates similar to those reported in human studies were observed, suggesting that the PDX model is clinically relevant for evaluating the efficacy of anticancer drugs [22,25–28]. A remarkable correlation between drug activity in PDXs and clinical outcome was reported when patients with advanced cancer were treated with selected regimens based on the treatment responses of their PDX [29,30], suggesting that PDXs could provide robust models for identifying effective treatment for cancer patients and for predicting clinical efficacy of drug candidates. Consequently, PDXs derived from various types of cancers have been reported recently, including those established from lung cancer [23,26,28,31]. Those studies have demonstrated the feasibility of using PDXs for translational studies in drug development, for molecular characterization of cancer biology, and for strategic development of individualized therapy. Nevertheless, few molecularly-annotated lung cancer PDXs are reported in literature and are not readily available for preclinical studies.

Our purpose here was to develop molecularly annotated PDXs for evaluation of investigational anticancer agents and mechanistic characterization of lung cancers. We established PDXs from surgical specimens of lung cancer patients and characterized the gene mutations in those PDXs and the corresponding primary tumors. Our results show that some novel genes were frequently mutated in primary lung cancers and that the mutations in primary tumors can be recapitulated by their corresponding PDX.

## Materials and methods

### *Human lung tissue specimens*

Fresh lung cancer samples were collected in 2012 and 2013 from surgically resected specimens under approved research protocols with informed consent from the patients. This study was approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center.

### *Generation of patient-derived xenografts in immune-defective mice*

All animal experiments were carried out in accordance with *Guidelines for the Care and Use of Laboratory Animals* (NIH publication number 85-23) and the institutional guidelines of MD Anderson Cancer Center. Six- to eight-week-old immune-defective non-obese or diabetic severe combined immunodeficiency (NOD-SCID) mice were obtained from Jackson Laboratory (Bar Harbor, Maine) or Charles River Laboratories, Inc. (Wilmington, MA).

Fresh surgical specimens of lung cancer were cut to about 2 $mm^3$ in size, briefly soaked in matrigel, and implanted into the flank subcutaneous space of mice (2 or 3 mice/patient specimen), as described elsewhere within 1 hour of surgical resection [32]. The mice were monitored for up to 10 months for tumor growth. The tumors were harvested when they reached 1.5 cm in diameter. The tumors (labeled F1 for the first passage in animals) were divided into 2–3 $mm^3$ specimens which were frozen in liquid nitrogen for future investigation, analyzed for molecular biological characterization, or reimplanted into mice to generate more tumorgrafts (F2, F3, etc., for subsequent passages).

### *Whole-exome sequencing for 202 cancer-related genes*

Genomic DNA was isolated from primary tumor tissues and PDX tissues by proteinase K digestion and phenol extraction. The whole-exome sequencing for 202 cancer-related genes is shown in Supplement Table S1. Briefly, DNA samples were quantified by Qubit (Invitrogen, Grand Island, NY) and their quality was assessed using Genomic DNA Tape for the 2200 Tapestation (Agilent, Santa Clara, CA). DNA from each sample was sheared by sonication using an E220 instrument (Covaris, Woburn, MA). To ensure the proper fragment size, samples were checked on the TapeStation using the DNA High Sensitivity kit (Agilent). The sheared DNA proceeded to library preparation with the KAPA library preparation kit (KAPA Biosystems, Wilmington, MA) following the manufacturer's protocol. Samples were quantified using the KAPA qPCR quantification kit. Equimolar amounts of DNA were pooled for capture (8–12 samples per pool).

After library preparation, 202 genes predicted to be clinically relevant in cancer were selected for capture. Biotin-labeled probes were designed with Roche Nimblegen for capturing all exons in the 202 genes, following the manufacturer's protocol for the capture step. The cutoff for enrichment was 50-fold minimum. The captured libraries were sequenced on a HiSeq 2000 (Illumina Inc., San Diego, CA) on a version 3 TruSeq paired end flow cell according to the manufacturer's instructions at a cluster density between 700 and 1000 K clusters/$mm^2$. Sequencing was performed on a HiSeq 2000 for $2 \times 100$ paired end reads with a 7-nt read for indexes using Cycle Sequencing v3 reagents (Illumina). The resulting BCL files containing the sequence data were converted to ".fastq.gz" files, and individual libraries within the samples were demultiplexed using CASAVA 1.8.2 software with no mismatches.

### *Data analysis*

We aligned the T200 target-capture deep-sequencing data to human reference assembly hg19 by using Burrows–Wheeler Aligner software [33] and removed duplicated reads by using SAMtools [34] (both Sourceforge open source software, Slashdot Media, San Francisco, CA). We called single nucleotide variants and small insertions/deletions by using VarScan2 [35] and called copy number alterations by using a previously published algorithm [36] that reports gain or loss status of each exon. Genomic DNA from the SCID mouse was used to exclude nucleotide variants observed in the mouse genome. To ensure specificity, variants with an allele frequency less than 10% were not reported. To understand the potential functional consequence of detected variants, we compared them with the dbSNP, COSMIC [37], and TCGA databases and annotated them using SIFT [38], Polyphen [39], Condel [40], and Mutation Assessor [41].

## Results

### *Establishing patient-derived xenografts from lung cancer specimens*

We collected surgically resected tumor samples from 88 NSCLC patients and implanted each specimen into 2–3 NOD-SCID mice to develop PDXs. We obtained 23 PDXs (Table 1). The overall implantation rate for development of a PDX was 26%. Squamous cancer and neuroendocrinal carcinoma had relatively higher implantation rates than adenocarcinoma. Moderately and poorly differentiated tumors had relative high implantation rates than well differentiated tumors (Fig. 1A). Nevertheless, the difference among those groups was not statistically significant ($P = 0.09–0.35$). The time from inoculation of the surgical specimen until harvest of the first generation of PDX (1.5 cm in diameter) ranged from 2 to 10 months, with an average of 4 months. The tumor engraftment rate is comparable to the rate reported for establishment of subcutaneous PDXs in SCID mice [31] but lower than that reported for engraftment from tumor specimens implanted under the renal capsule [26]. The tumors were harvested when they reach 1.5 cm in diameter. The tumors (labeled as F1 for the first passage in animals) were divided into several portions of about 2–3 $mm^3$, which were frozen in liquid