Drug-sensitive FGFR3 mutations in lung adenocarcinoma

P. Chandrani1,2†, K. Prabhash2,3†, R. Prasad1, V. Sethunath1, M. Ranjan1, P. Iyer1,2, J. Aich1, H. Dhamne1, D. N. Iyer1, P. Upadhyay1,2, B. Mohanty4, P. Chanda5, R. Kumar6, A. Joshi3, V. Noronha3, V. Patil3, A. Ramaswamy3, A. Karpe3, R. Thorat6, P. Chaudhari4, A. Ingle7, A. Choughule3 & A. Dutt1,2*

1Integrated Genomics Laboratory, Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Navi Mumbai; 2Homi Bhabha National Institute, Training School Complex, Anushakti Nagar, Mumbai; 3Department of Medical Oncology, Tata Memorial Hospital; 4Small Animal Imaging Facility, Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Navi Mumbai; 5AceProbe Technologies Pvt. Ltd, New Delhi, India; 6Department of Pathology, Tata Memorial Hospital; 7Laboratory Animal Facility, Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Navi Mumbai

†Both authors contributed equally to this work.

*Correspondence to: Dr Amit Dutt, Wellcome Trust/DBT India Alliance Intermediate Fellow, Tata Memorial Centre, ACTREC, Navi Mumbai 410 210, India. Tel: +91-22-27405056; E-mail: adutt@actrec.gov.in

Background: Lung cancer is the leading cause of cancer-related deaths across the world. In this study, we present therapeutically relevant genetic alterations in lung adenocarcinoma of Indian origin.

Materials and methods: Forty-five primary lung adenocarcinoma tumors were sequenced for 676 amplicons using RainDance cancer panel at an average coverage of 1500 \( \times \) (reads per million mapped reads). To validate the findings, 49 mutations across 23 genes were genotyped in an additional set of 363 primary lung adenocarcinoma tumors using mass spectrometry. NIH/3T3 cells over expressing mutant and wild-type FGFR3 constructs were characterized for anchorage independent growth, constitutive activation, tumor formation and sensitivity to FGFR inhibitors using in vitro and xenograft mouse models.

Results: We present the first spectrum of actionable alterations in lung adenocarcinoma tumors of Indian origin, and shows that mutations of FGFR3 are present in 20 of 363 (5.5%) patients. These FGFR3 mutations are constitutively active and oncogenic when ectopically expressed in NIH/3T3 cells and using a xenograft model in NOD/SCID mice. Inhibition of FGFR3 kinase activity inhibits transformation of NIH/3T3 overexpressing FGFR3 constructs and growth of tumors driven by FGFR3 in the xenograft models. The reduction in tumor size in the mouse is paralleled by a reduction in the amounts of phospho-ERK, validating the in vitro findings. Interestingly, the FGFR3 mutations are significantly higher in a proportion of younger patients and show a trend toward better overall survival, compared with patients lacking actionable alterations or those harboring KRAS mutations.

Conclusion: We present the first actionable mutation spectrum in Indian lung cancer genome. These findings implicate FGFR3 as a novel therapeutic in lung adenocarcinoma.

Key words: lung adenocarcinoma, actionable mutations, fibroblast growth factor receptor 3, oncogene, FGFR inhibitors, mass spectrometry

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide, accounting for over a million deaths annually [1]. Molecularly targeted therapies improve outcome for lung adenocarcinoma patients whose tumors harbor mutant EGFR or translocated ALK, RET or ROS1, with an encouraging response for those with mutated BRAF, MET, NTRK-1 & 2 and ERBB2 [2–5]. Such oncogenic somatic alterations though vary across populations/ethnic groups, e.g. EGFR mutations are present in over 30% of East Asian lung adenocarcinoma patients, however, they are only found in ~23%–25% of Indian and 10% of Western lung adenocarcinoma patients [6–8]. Similarly, KRAS mutations are present at 60% lower frequency in Indian lung adenocarcinoma patients than compared with the Caucasian population [3, 9, 10].
The diversity in somatic alterations lends similarity to the known plurality in clinical response based on ethnicity and divergent genetic and environmental factors [11]. Thus, besides the unmet need for additional therapeutic targets in lung adenocarcinoma patients, it is equally pertinent to profile known oncogenic somatic alterations across different populations to understand their landscape of variability.

Here, in an attempt to profile for activating alterations, we have generated a comprehensive mutational spectrum of activating alterations prevalent among lung adenocarcinoma patients of Indian origin, considered to be an admixture population of non-European Caucasian and Ancestral South Indians. We also report the first incidence of activating and drug sensitive FGFR3 mutations in lung adenocarcinoma. FGFR3 mutated samples, with ~5% population frequency, form a distinct subclass apart from EGFR, KRAS and EML4-ALK.

Materials and methods

Patients

To profile for therapeutically relevant genome alterations in lung adenocarcinoma of Indian origin, FFPE blocks with known EGFR mutation status for 45 consecutive histologically confirmed lung adenocarcinoma patients tumor samples (stage IV, 49% males and 51% non-smokers) for sequencing and an additional set of 363 consecutive lung adenocarcinoma patients tumor samples (stage IV, 62% males and 73% non-smokers) for mass spectrometry were retrospectively collected from Tata Memorial Hospital (supplementary Table S1, available at Annals of Oncology online).

Pooling of samples, target gene-capturing and next generation sequencing

A set of 45 lung adenocarcinoma samples were sequenced using pooled sequencing approach to capture low-frequency variants [12–14]. Briefly, 45 samples were divided into duplicate pools of different population sizes (supplementary Figure S1, available at Annals of Oncology online), i.e. 2 pools of 5 individuals (5AX and 5XB), 2 pools of 10 individuals (10XA and 10XB) and 1 pool of 15 individuals (15X) for next-generation sequencing (NGS) of 676 genomic regions of 158 genes as described earlier [15].

Discovery of genomic variants using computational analysis

FASTQ files were analyzed using BWA-Picard-GATK/MuTect pipeline generating 3349 unique variants (supplementary Table S2, available at Annals of Oncology online), Polymorphisms overlapping with dbSNP database (v.142) and Indian specific SNP database TMC-SNPdb derived from whole exome sequencing of 62 normal samples [16] were filtered (supplementary Figures S2 and S3, available at Annals of Oncology online). Stringent mutation analysis was carried out as further detailed in supplementary methods, available at Annals of Oncology online to derive list of significant mutations for further validation (supplementary Tables S2 and S3, available at Annals of Oncology online).

Mass spectrometry based genotyping

Briefly, PCR and extension primers for 49 mutations in 23 genes were designed using single base extension based mass spectrometry assay design 3.1 software (supplementary Table S4, available at Annals of Oncology online). Mutation calls were analyzed using Typer 4 (Sequenom, Inc., USA) and were reviewed by manually observing mass spectra.

Cell culture, anchorage-independent growth assay and immunoblotting

NIH/3T3 cells transduced with FGFR3 wild-type and mutant construct were used for induction and drug inhibition study as described in supplementary methods, available at Annals of Oncology online. Anchorage independent growth assay and immunoblotting were carried out as described earlier [17], and as detailed in the supplementary methods, available at Annals of Oncology online.

Xenograft development

A cohort of eight NOD-SCID or nude mice per clone were subcutaneously injected with five million cells for tumor formation in 2–3 months. Inhibitor BGJ-398 [18] was given at 15 and 30 mg/kg along with vehicle control (10% Tween-80) independently to randomized xenograft groups after tumor size reaching ~150 mm^3. Tumor size was measured every alternate day using a Vernier caliper during the 14 day period of drug treatment. microPET-CT scan was carried out at the end of the drug treatment.

Immunohistochemistry

Immunohistochemistry analysis was carried out as described earlier [19] and detailed in supplementary methods, available at Annals of Oncology online.

Overall survival analysis

Overall survival (OS) of patients was assessed using Kaplan–Meier method using R and IBM SPSS software package, as detailed in supplementary methods, available at Annals of Oncology online. The end point was taken as date of death with censoring implied at the date of the last contact.

Results

Recurrent FGFR3 mutations in lung adenocarcinoma patient of Indian origin

To identify low frequency and ethnic-specific therapeutically relevant genome alterations in lung adenocarcinoma of Indian origin, we sequenced 45 primary lung adenocarcinoma stage IV tumors (supplementary Table S1, available at Annals of Oncology online) for 676 amplicons at an average coverage of 1500× (reads per million mapped reads), as described in supplementary Figures S1–S5 and Tables S2, S3, available at Annals of Oncology online. To validate the findings, we selected 49 mutations occurring across 23 genes based on their recurrence and therapeutic significance (supplementary Table S4, available at Annals of Oncology online), for genotyping in an additional set of 363 primary lung adenocarcinoma stage IV tumors (Figure 1A; supplementary Table S5, available at Annals of Oncology online) using mass spectrometry.

Based on the mutation profiling of 363 lung adenocarcinoma patients, we present the first portrait of activating mutations present in the Indian lung cancer genome (Figure 1B), wherein 160 of 363 patients were found to harbor activating mutations across 8 genes at following frequency: EGFR (28.4%), KRAS (13%), ALK (3.8%), AKT1 (2.5%), PIK3CA (1.4%), FGFR4 (0.4%) and ERBB2 (0.3%) as shown in Figure 1A, consistent with earlier reports [6, 8, 9]. In addition, 73 of 369 patients were found to harbor EML4-ALK translocation as determined by FISH. Among the other most significantly mutated genes, we found recurrent FGFR3 mutations in 20 of 363 tumors (5.5%), of which 7 co-occurred in samples harboring EGFR (n = 5) and KRAS (n = 2) mutations. In total, 16 patients harbored FGFR3 (S249C) mutation; and 4 patients...
harbored a novel FGFR3 (G691R) mutation (Figure 1A and C, upper panel; supplementary Figure S6 and Table S6, available at Annals of Oncology online).

Interestingly, FGFR3 (S249C) mutation has previously been described as activating and drug sensitive in lung squamous [20], while the novel FGFR3 (G691R) mutation was predicted to be deleterious based on using 7 of 10 functional prediction tools (supplementary Table S3, available at Annals of Oncology online).

FGFR3 mutations in lung adenocarcinoma are activating in vitro and in vivo

To test whether the recurrent FGFR3 mutations found in this study are activating we transduced NIH/3T3 fibroblast cells with retroviruses encoding the FGFR3 G691R mutation along with WT FGFR3 and the previously characterized FGFR3 (R248C) and

\[
\begin{array}{c|c|c|c}
\text{NIH3T3 cells transfected with} & \text{Total number of} & \text{Total number of mice showing tumor formation} \\
\text{FGFR3} & \text{mice injected} & \\
\hline
\text{WT} & 11 & 3 \\
\text{G691R} & 12 & 6 \\
\text{S249C} & 12 & 6 \\
\text{R248C} & 11 & 3 \\
\end{array}
\]
(S249C) mutations [20]. Similar to FGFR3 R248C and S249C, the ectopic expression of the novel G691R mutant clone in pooled NIH/3T3 cells conferred anchorage-independent growth, forming threefold more colonies in soft agar than cells expressing WT FGFR3 (Figure 1C, left panel), despite higher expression levels of WT FGFR3 (Figure 1C, right panel). The transformation was accompanied by elevated phosphorylation of the downstream ERK1/2 and AKT1 in a constitutive manner (Figure 1D). Furthermore, consistent with the in vitro data, NIH/3T3 cells expressing transforming FGFR3 mutations or WT when injected subcutaneously into NOD/SCID mice formed tumors within 2 months post injection of cells. While 3 of 11 mice injected with FGFR3 WT formed tumors, 12 of 12 mice injected with cells expressing FGFR3 S249C; and 6 of 12 mice injected with cells expressing FGFR3 G691R formed tumors (Figure 1E). The tumor size doubling time was ∼7 days for cells expressing FGFR3 (G691R), ∼5 days for cells expressing FGFR3 (S249C); the FGFR3-WT tumors doubled in size in ∼9–10 days.

FGFR3 mutations in lung adenocarcinoma are sensitive to inhibitors in vitro and in vivo

We further show that inhibition of FGFR3 kinase activity using pan FGFR inhibitor PD173074 block the constitutive phosphorylation of ERK1/2 (Figure 2A). Similarly, the treatment of cells harboring activating FGFR3 mutations with PD173074 result in a marked decrease in colony formation in soft agar and cell survival in liquid culture (Figure 2B). Extending the effect in vitro studies, when tumors reached ∼100–200 mm² in all mice injected with NIH/3T3 cells began treatment with 15 or 30 mg/kg BGJ398—a selective FGFR inhibitor currently under clinical trials for various cancer types (as PD173074 is incompatible with in vivo studies [21]), or vehicle for 14 days. Tumors treated with BGJ398 slowed or reversed their growth compared with vehicle (Figure 2C, upper panel), so that by the end of the study, the effect on tumor burden in vehicle-treated versus BGJ398-treated mice were 3.3-folds in FGFR3 (S249C), three-fold in FGFR3 (G691R) and 2.25-fold in FGFR3-WT xenografts (Figure 2D). This reduction in tumor size was paralleled by a reduction in the amounts of phospho-ERK1/2 in immuno-histochemical analyses (Figure 2C, lower panel) of explanted tumors, validating our in vitro findings that MAPK signaling is the key pathway engaged by mutated FGFR3.

Correlation of FGFR3 mutations with clinicopathological features of lung cancer patients

Clinically, lung adenocarcinoma patients with FGFR3 mutation positive tumors expressing higher activated MAPK levels (supplementary Figure S7, available at Annals of Oncology online) show a better trend in OS with 17 months (n = 8; 95% CI: 6.4–27.5; HR: 0.6) compared with 14 months (n = 197; 95% CI: 8.7–13.2) in patients with wild-type FGFR3 (Figure 2E). The OS trend in lung adenocarcinoma patients though is similar to bladder urothelial carcinomas and skin cutaneous melanoma patients, but not to head and neck cancer and lung squamous carcinoma patients, based on our analysis using cBioPortal for survival of patients harboring activating FGFR3 mutations in different cancers (supplementary Figure S8, available at Annals of Oncology online). Furthermore, the FGFR3 mutations were observed to be significantly higher in patients <45 years (9 of 95) than in patients >45 years (11 of 269) (P = 0.048) but not with their gender and smoking status (supplementary Table S7, available at Annals of Oncology online). The sample size in this study, however, is underpowered to reach statistical significance for survival data.

Discussion

We present the first portrait of clinically actionable alterations in lung adenocarcinoma of Indian origin that includes EGFR, KRAS, EML4-ALK, AKT1, PIK3CA, FGFR4 and ERBB2, similar to that identified in other ethnic groups [5, 22, 23], and an additional subset of patients with FGFR3 mutations. Ethnic-specific variations have been well known in lung cancer [24, 25] across different populations. We observed 28.4% EGFR mutations and 13% KRAS mutations in lung adenocarcinoma patients, consistent with our previous report [6, 9]. Similarly, variation in frequency of other molecular alterations is also observed such as 3% EML4-ALK alteration in our study compared with 8% in Caucasian population [3] and in 5% Chinese population [23]. ERBB2 mutation found at <1% frequency in our cohort exists at ∼2%–3% among the Caucasian [3] and Chinese populations [23]. Similarly, AKT1 mutations were found at higher than the reported <1% in both Caucasian [3] and Chinese populations [23] indicating the higher therapeutic relevance of AKT1 targeted compounds in Indian population.

We have also identified frequent and recurrent drug sensitive FGFR3 mutations in lung adenocarcinoma patients. Among the Caucasians, activating mutations in FGFR3 have been earlier reported in bladder carcinoma [26], lung squamous cell carcinomas [20] and cervical cancer [27], but were found to be largely absent in lung adenocarcinomas [23, 28, 29], except for Imielinski et al. who reported non-recurrent somatic FGFR3 mutations of unknown functional significance in 3 of 183 lung adenocarcinoma patients [10]. On the other hand, the presence of frequent FGFR3 mutations (with unknown driving potential) is tangentially referred to in the literature among Korean lung adenocarcinomas patients [30]. Along with these reports, our finding of activating FGFR3 mutations in lung adenocarcinoma patients provides an interesting convergence with mouse genetic experiments wherein activated FG9-FGFR3 signal acts as the primary oncogenic pathway involved in initiation of lung adenocarcinoma [31, 32].

Analyzing the potential effect of FGFR3 driver mutations on survival of lung cancer patients, we observed a trend toward better survival for FGFR3 mutations in lung adenocarcinoma, compared with lung adenocarcinoma patients with wild-type FGFR3 and those harboring KRAS mutation, similar to as reported in the bladder and skin cancer [33]. Thus, FGFR3 mutation represents an opportunity for targeted therapy in lung adenocarcinoma. FGFR inhibitors, which are currently in clinical testing in tumor types bearing genetic alterations in FGFR genes [34, 35], may be extended to evaluate in patients with FGFR3-mutated lung adenocarcinoma. Finally, with a broader emerging role across different cancers [20, 36–39], this study further underscores that FGFR family may potentially join the EGFR family as a widespread target for therapeutic intervention in several human cancers.
Figure 2. Transformed NIH/3T3 cells and xenografts are sensitive to FGFR inhibitor. (A) Immunoblot analysis of NIH/3T3 clones treated with FGF1 (50 ng/ml) followed by FGFR inhibitor PD173074 (2 μM) is shown. GAPDH was used as loading control. (B) Soft agar colony count (averaged from 3 replicates) and IC-50 values of NIH/3T3 clones expressing wild-type or mutant FGFR3, treated with increasing concentration of PD173074 is shown. (C) Upper panel: NIH/3T3 xenografts developed into NOD-SCID mice were treated with FGFR inhibitor BGJ-398 or vehicle for 21 days. CT-scan and a readout for relative 18F-FDG uptake is shown by a gradient color code with red indicating as maximum uptake. Lower panel: Immuno-histochemical staining of total and phospho-ERK1/2 is shown in xenografts treated with drug and vehicles. (D) The plot shows tumor size (normalized to the size at day 0 of drug treatment) during the course of drug treatment indicating a reduced tumor size in drug-treated xenografts. (E) Clinical follow-up of total 205 patients for up to 62 months was used for the Kaplan–Meier analysis. 

EGFR positive patients received Gefitinib as a regular therapeutic regimen while rest of the patients received conventional chemotherapy. The table below the plot indicates patients at risk during the course of 60 months and median survival for each mutant group. Detailed figure legend can be found in supplementary materials, available at Annals of Oncology online.
Acknowledgements

All members of the Dutt laboratory for critically reviewing the manuscript. RainDance technologies, Inc. for providing NGS library preparation services. Sandor LifeSciences Pvt. Ltd for providing NGS services. AD is supported by an Intermediate Fellowship from the Wellcome Trust/DBT India Alliance (IA/I/11/2500278), by a grant from DBT (BT/PR2372/AGR/36/696/2011), from Terry Fox Foundation through TMC-Research Administrative Council (TRAC; project 108); and intramural grants (IRB project 92 and 55). PC is supported by a senior research fellowship from CSIR. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Funding

This work was supported by Wellcome Trust/Department of Biotechnology India Alliance [IA/I/11/2500278], Department of Biotechnology, Government of India [BT/PR2372/AGR/36/696/2011], and intramural grants [IRB project 92, 55 and 108].

Disclosure

The authors have declared no conflicts of interest.

References

1. Society AC, Cancer Facts & Figures 2012. Atlanta: American Cancer Society 2012.
2. Barlesi F, Blons H, Beau-Faller M et al. Biomarkers (BM) France: Results of routine EGFR, HER2, KRAS, BRAF, PI3KCA mutations detection and EML4-ALK gene fusion assessment on the first 10,000 non-small cell lung cancer (NSCLC) patients (pts). J Clin Oncol 2013; 31: 486s.
3. Kris MG, Johnson BE, Berry LD et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA 2014; 311: 1998–2006.
4. Tsao AS, Scagliotti GV, Bunn PA, Jr et al. Scientific advances in lung cancer 2015. J Thorac Oncol 2016; 11: 613–638.
5. Campbell JD, Alexandrov A, Kim J et al. Distinct patterns of somatic mutations in non-small cell lung cancer (NSCLC) patients (pts) with mutant but not wild-type EGFR predicts response to tyrosine-kinase inhibitors in human lung cancer. Br J Cancer 2014; 111: 2203–2204.
6. Chougule A, Prabhask K, Noronha V et al. Frequency of EGFR mutations in 907 lung adenocarcinoma patients of Indian ethnicity. PLoS ONE 2013; 8: e76164.
7. Lynch TJ, Bell DW, Sordella R et al. Activating mutations in the epidermal growth factor receptor underlining responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004; 350: 2129–2139.
8. Noronha V, Prabhask K, Thavamani A et al. EGFR mutations in Indian lung cancer patients: clinical correlation and outcome to EGFR targeted therapy. PLoS ONE 2013; 8: e61561.
9. Chougule A, Sharma R, Trivedi V et al. Coexistence of KRAS mutation with mutant but not wild-type EGFR predicts response to tyrosine-kinase inhibitors in human lung cancer. Br J Cancer 2014; 111: 2203–2204.
10. Imielinski M, Berger Alice H, Hammerman PS et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell 2012; 150: 1107–1120.
11. Patel JN. Cancer pharmacogenomics: implications on ethnic diversity and drug response. Pharmacogenet Genomics 2015; 25: 223–230.
12. Chauhan R, Warner JB, Nakano M et al. Microdroplet-based PCR enrichment for large-scale targeted sequencing. Nat Biotechnol 2009; 27: 1025–1031.
13. Niranjan TS, Adamczyk A, Bravo HG et al. Effective detection of rare variants in pooled DNA samples using Cross-pool tailcurve analysis. Genome Biol 2011; 12: R93.
14. Kaartokallio T, Wang J, Heinonen S et al. Exome sequencing in pooled DNA samples to identify maternal pre-eclampsia risk variants. Sci Rep 2016; 6: 29085.
15. Harismendy O, Schwab RB, Bao L et al. Detection of low prevalence somatic mutations in solid tumors with ultra-deep targeted sequencing. Genome Biol 2011; 12: R124.
16. Upadhay P, Gardi N, Desai S et al. TMC-SNPdb: an Indian germline variant database derived from whole exome sequences. Database (Oxford) 2016; 2016.
17. Chandrani P, Upadhay P, Iyer P et al. Integrated genomics approach to identify biologically relevant alterations in fewer samples. BMC Genomics 2015; 16: 936.
18. Guagnano V, Furet P, Spanka C et al. Discovery of 3-(2,6-dichloro-3,5-dimethoxy-phenyl)-1-[6-[4-(4-ethyl-piperazin-1-yl)-phenylamin o]-pyrimidin-4-yl]-1-methyl-urea (NVP-BGJ398), a potent and selective inhibitor of the fibroblast growth factor receptor family of receptor tyrosine kinase. J Med Chem 2011; 54: 7066–7083.
19. Upadhay P, Nair S, Kaur E et al. Notch pathway activation is essential for maintenance of stem-like cells in early tongue cancer. Oncotarget 2016; 7: 50437–50449.
20. Liao RG, Jung J, Tchaicha JH et al. Inhibitor-sensitive FGFR2 and FGFR3 mutations in lung squamous cell carcinoma. Cancer Res 2013; 73: 5195–5205.
21. Touat M, Ileana E, Postel-Vinay S et al. Targeting FGFR signaling in cancer. Clin Cancer Res 2013; 21: 2684–2694.
22. Barlesi F, Mazieres J, Merlio JP et al. Routine molecular profiling of patients with advanced non-small-cell lung cancer: results of a 1-year nationwide programme of the French Cooperative Thoracic Intergroup (IFCT). Lancet 2016; 387: 1415–1426.
23. Wang R, Zhang Y, Pan Y et al. Comprehensive investigation of oncogenic driver mutations in Chinese non-small cell lung cancer patients. Oncotarget 2015; 6: 34300–34308.
24. Hardy D, Liu CC, Xia R et al. Racial disparities and treatment trends in a large cohort of elderly black and white patients with nonsmall cell lung cancer. Cancer 2009; 115: 2199–2211.
25. Bollig-Fischer A, Chen W, Gadgeel SM et al. Racial diversity of actionable mutations in non-small cell lung cancer. J Thorac Oncol 2015; 10: 250–253.
26. Tomlinson DC, Hurst CD, Knowles MA. Knockdown by shRNA identifies S249C mutant FGFR3 as a potential therapeutic target in bladder cancer. Oncogene 2007; 26: 5889–5899.
27. Rosty C, Aubriot MH, Cappellen D et al. Clinical and biological characteristics of cervical neoplasias with FGFR3 mutation. Mol Cancer 2005; 4: 15.
28. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. Nature 2014; 511: 543–550.
29. Helsten T, Elkin S, Arthur E et al. The FGFR landscape in cancer: analysis of 4,853 tumors by next-generation sequencing. Clin Cancer Res 2016; 22: 259–267.
30. Lim SM, Kim HR, Cho EK et al. Targeted sequencing identifies genetic alterations that confer Primary resistance to EGFR tyrosine kinase inhibitor of the fibroblast growth factor family of receptor tyrosine kinase. J Med Chem 2011; 54: 7066–7083.
31. Patel JN, Cancer pharmacogenomics: implications on ethnic diversity and drug response. Pharmacogenet Genomics 2015; 25: 223–230.
32. Chauhan R, Warner JB, Nakano M et al. Microdroplet-based PCR enrichment for large-scale targeted sequencing. Nat Biotechnol 2009; 27: 1025–1031.
33. Liu X, Zhang W, Geng D et al. Clinical significance of fibroblast growth factor receptor-3 mutations in bladder cancer: a systematic review and meta-analysis. Genet Mol Res 2014; 13: 1109–1120.

34. Milowsky MI, Dittrich C, Duran I et al. Phase 2 trial of dovitinib in patients with progressive FGFR3-mutated or FGFR3 wild-type advanced urothelial carcinoma. Eur J Cancer 2014; 50: 3145–3152.

35. Sequist LV, Cassier P, Varga A et al. Phase I study of BGJ398, a selective pan-FGFR inhibitor in genetically preselected advanced solid tumors. In American Association for Cancer Research 2014 Congress. San Diego, CA, USA 2014.

36. Dutt A, Ramos AH, Hammerman PS et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. PLoS ONE 2011; 6: e20351.

37. Dutt A, Salvesen HB, Chen TH et al. Drug-sensitive FGFR2 mutations in endometrial carcinoma. Proc Natl Acad Sci USA 2008; 105: 8713–8717.

38. Weiss J, Sos ML, Seidel D et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med 2010; 2: 62ra93.

39. Dienstmann R, Rodon J, Prat A et al. Genomic aberrations in the FGFR pathway: opportunities for targeted therapies in solid tumors. Ann Oncol 2014; 25: 552–563.