

# Oncogene alterations in non-small cell lung cancer with *FGFR1* amplification—novel approach to stratify patients who benefit from FGFR inhibitors

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Lung cancer is the principal cause of cancer-related deaths worldwide (1). The discovery of driver oncogenes in non-small cell lung cancer (NSCLC) has revolutionized treatment for this disease. Therapies to driver alterations such as mutations in *EGFR*, *BRAF*, and *ROS1*, and translocation of the *ALK*, are now available in clinical practice. The appropriate implementation of targeted therapies has significantly prolonged the survival of NSCLC patients with driver oncogenes (2,3). In contrast to the significant progress achieved in targeted therapy for lung adenocarcinoma, the identification and targeting of driver mutations in squamous cell lung cancer (SQCLC) remains to be established.

One potential molecular target in SQCLC is the fibroblast growth factor receptor 1 (FGFR1). In SQCLC, *FGFR1* amplification is detected in approximately 13–22% of patients (4–6). The FGFR pathway plays critical roles in cell migration, mitosis, and death, suggesting a role for this network in the oncogenic process (7). Deregulation

of FGFR signaling has been reported in a variety of human cancers such as breast and bladder, and FGFR inhibitors have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of *FGFR2*-fusion cholangiocarcinoma and *FGFR3*-mutated urothelial carcinoma (8). In contrast to the demonstrated efficacy of these agents in targeting *FGFR* fusions/mutations in other types of cancers, FGFR inhibitors show only a modest response in *FGFR1* amplified SQCLC (9–11). As such, there is a clear need to elucidate the mechanisms underlying the heterogeneous response of SQCLC to FGFR inhibitors, and to establish markers that predict response to these agents.

The driver role of *FGFR1* amplification could be affected by neighboring genes in the 8p11–12 locus (12). The 8p12 region includes multiple centers of amplification with chromosomal breakpoints spanning an unusually large region surrounding the peak (13). Therefore, *FGFR1* amplification is associated with many other genes located

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in the 8p11–12 locus, such as *BAG4*, *NSD3* (*WHSC1L1*), *LSM1*, *ASH2L*, *DDHD2*, and *EIF4EBP1*. In one study, only 28% of cases with 8p12 amplification had peaks that centered on *FGFR1*, raising the possibility that *FGFR1* is not the driver in the majority of cases with 8p12 amplification (13). In line with this, other genes located in the 8p11–12 locus were also reported to contribute to oncogenesis (14). For example, *NSD3* was identified as an alternative driver in *FGFR1*-amplified tumors (15). *NSD3* is a lysine methyltransferase involved in various cellular processes, including transcriptional regulation and DNA damage repair (16–18). Yuan *et al.* identified that suppression of *NSD3*, but not of *FGFR1*, diminished tumor growth and prolonged survival in a mouse model of SQCLC (19). They also showed that knockout of *NSD3* in patient-derived xenografts from primary SQCLC with *NSD3* amplification attenuated tumor growth *in vivo*.

In addition to its genetic heterogeneity, the expression of *FGFR1* messenger RNA (mRNA) is not well correlated with the magnitude of *FGFR1* gene amplification. Kotani *et al.* showed that there is a discrepancy between the genetic amplification and protein expression of *FGFR1* in *FGFR1*-amplified SQCLC cell lines. While cell lines with high *FGFR1* amplification and protein expression are sensitive to *FGFR* inhibitor monotherapy, activation of additional driver oncogenes such as *HER2* and *PDGFR* mitigate the effect of *FGFR1* inhibition in *FGFR1* amplified but low protein-expressing cells (20). In addition, among 25 patients with *FGFR1* amplified SQCLC identified by fluorescence in situ hybridization (FISH) analysis, only six tumors were positive for *FGFR1* protein expression. Similarly, Wynes *et al.* demonstrated that sensitivity to the *FGFR* inhibitor ponatinib [half-maximal inhibitory concentration ( $IC_{50}$ ) value  $\leq 50$  nM] was correlated with *FGFR1* mRNA and protein expression, but not *FGFR1* gene copy number in lung cancer cell lines (21). Consistent with preclinical findings, *FGFR1* protein expression was only detected in 35.5% of *FGFR1* amplified cases, while *FGFR1* protein expression was also detected in 29.6% of non-amplified cases among 208 SQCLC tumor samples (22). Likewise, in a phase II study with the *FGFR* inhibitor dovitinib, among 16 patient samples with *FGFR1* amplification, 31.2% showed medium/high expression, while 43.7% showed low expression, and 25% showed no *FGFR1* mRNA expression (11). Furthermore, this study did not demonstrate a correlation between *FGFR1*–3 mRNA level and response to dovitinib, although the sample size was limited. These data indicate that *FGFR1* amplification is not indicative of

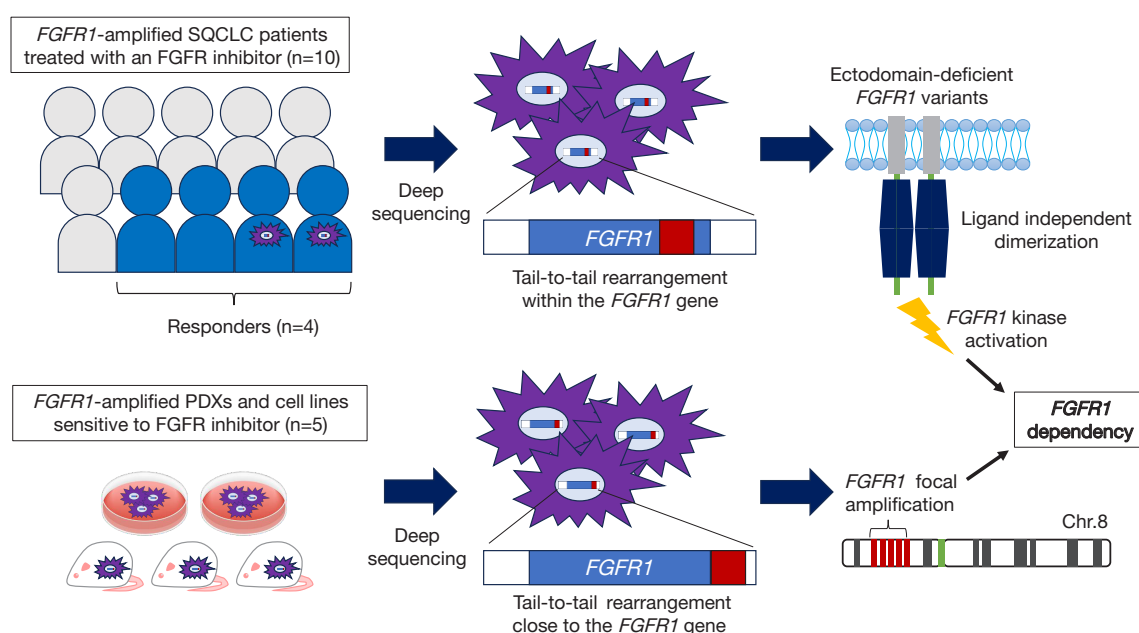
its mRNA or protein expression, and even high *FGFR1* protein expression may not be enough to explain the efficacy to *FGFR* inhibitors.

Recently, Malchers *et al.* demonstrated that there's a correlation between the oncogenic ectodomain deletion of *FGFR1* caused by somatic rearrangements with tumor dependency on *FGFR1* and sensitivity to *FGFR* inhibitors in SQCLC (23). By performing deep genomic sequencing of SQCLC samples with *FGFR1* amplification, including patient samples treated with *FGFR* inhibitors, they identified two types of tail-to-tail rearrangements that happen within or close to the *FGFR1* gene (Figure 1).

Tail-to-tail rearrangements within the *FGFR1* gene were identified in 8% of *FGFR1* amplified samples. Although tail-to-tail rearrangements occur within the open reading frame, the truncated *FGFR1* protein can still be transcribed using a noncanonical in-frame ATG start codon, resulting in various deletions in the extracellular region of the protein. The loss of ectodomain components causing *FGFR1* dependence is presumably mediated by the enhancement of ligand-independent dimerization, leading to sensitivity to *FGFR* inhibitors. Interestingly, half of the patients who responded to *FGFR* inhibitors harbored *FGFR1* amplification due to tail-to-tail rearrangements within the *FGFR1* gene, while these rearrangements were not detected in non-responders.

Tail-to-tail rearrangements were also found in the locus that is close to the *FGFR1* gene. These rearrangements led to an *FGFR1*-centered amplification pattern, and they frequently co-existed with disruptive rearrangements of *NSD3*. Tail-to-tail rearrangements close to *FGFR1* with *NSD3*-disruptive rearrangements were found in the NCI-H1581 and DMS114 cell lines, both of which are well known to be sensitive to *FGFR* inhibitors. Furthermore, tail-to-tail rearrangements in or close to the *FGFR1* gene were detected in seven of nine cases in a collective analysis of cell lines, patient-derived xenograft models, and patient-derived specimens that were sensitive to *FGFR1* inhibitors.

Mechanistically, 8p11 amplification and *FGFR1* dependence were at least partly mediated by break-fusion-bridging (BFB), a mechanism that leads to complex genome rearrangements in multiple cancers (24). A BFB cycle begins with chromosomal rearrangements mediated by the foldback inversion of two sister chromatids due to a lack of telomeres during DNA replication, resulting in two centromeres in the fused bridge. These foldback inversions were mediated by formation of single-stranded DNA hairpins. In the anaphase, two centromeres are stretched



**Figure 1** Tail-to-tail rearrangements within or close to the *FGFR1* gene associated with FGFR1 dependency. FGFR1, fibroblast growth factor receptor 1; SQCLC, squamous cell lung cancer; PDX, patient-derived xenograft.

to opposite poles. Then, the two sister chromatids are split with a double-strand break on the bridge between the two centromeres. Since each daughter cell contains chromatids without telomeres, the BFB cycle can repeat. Repetition of BFB cycles contributes to a surge in stair-like copy number amplifications and foldback inversions. Given that BFB can affect a number of genes besides *FGFR1*, and tail-to-tail rearrangements can be induced by other mechanisms such as chromothripsis, these tail-to-tail rearrangements randomly happen, and their occurrence within or close to the *FGFR1* gene likely promote a survival advantage.

The study by Malchers *et al.* (23) suggests that the identification of tail-to-tail rearrangements within or close to the *FGFR1* gene could be a potential new approach to select patients for FGFR inhibitor treatment. However, *FGFR1* amplified SQCLC is still heterogenous. While two of the four patients who responded to FGFR1 inhibitors did not show tail-to-tail rearrangements within the *FGFR1* gene, rearrangements within or close to *FGFR1* were also observed in approximately 25% of the FGFR1 inhibitor-insensitive models. First, it would be important to know how much FGFR1 mRNA and protein is expressed in tumors harboring tail-to-tail rearrangements, as discrepancies between gene amplification and mRNA expression of FGFR1 have been reported. Second, the

real value of *FGFR1* amplification remains to be fully elucidated. In the case of tail-to-tail rearrangements within the *FGFR1* gene, the ectodomain-lacking FGFR1 caused FGFR1 oncogene dependency in Ba/F3 cells. In contrast, although tail-to-tail rearrangements upstream of *FGFR1* lead to *FGFR1*-centered amplification in the 8p11 locus, it is unclear whether its amplification is essential for tumor cell survival. Furthermore, tumors with tail-to-tail rearrangements close to *FGFR1* were accompanied by destructive rearrangements in *NSD3*. This phenomenon can potentially induce unfavorable conditions for tumor growth, given the proliferative role of *NSD3* (19).

A series of studies by the authors illustrates the complexity of SQCLC (13,23,25), probably due to tobacco smoking. It is hoped that continued research efforts will eventually build a targeted strategy for this difficult population.

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