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AUTHOR'S VIEWS

Breaking bad family ties: Pan-ERBB blockers inhibit KRAS driven lung tumorigenesis

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ABSTRACT

Oncogenic K-RAS mutations were believed to lock the molecular switch in the ON state, independent of upstream activation. However, we demonstrate in preclinical models that activity of mutated K-RAS depends on upstream signaling events involving EGF receptor family members. This finding reveals a potential therapeutic vulnerability using pan-ERBB inhibitors to fight K-RAS mutated lung tumors.

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Oncogenic kirsten rat sarcoma oncogene homolog (*KRAS*) mutations were believed to lock the molecular switch in the ON state, independent of upstream activation. However, we demonstrate in preclinical models that activity of mutated KRAS depends on upstream signaling events involving epidermal growth factor (EGF) receptor family members. This finding reveals a potential therapeutic vulnerability using pan-ERBB inhibitors to fight *KRAS* mutated lung tumors.

Despite the advent of targeted therapy and immunotherapy, lung cancer still remains the deadliest form of cancer.[1] In general, lung cancers are sub-classified into several subtypes based on morphology, and further subdivided according to the tumor driving oncogene. Overall, kirsten rat sarcoma oncogene homolog (*KRAS*) mutated lung adenocarcinomas (LUAD) represent the most common group of all lung cancers. Unfortunately, and in contrast to other frequently mutated oncogenes as for instance the epidermal growth factor receptor (*EGFR*) there are no direct inhibitors targeting mutant KRAS available in the clinics. Accordingly, research focused to identify up- or downstream signal mediators to reduce activity of mutated KRAS. In this context, initial clinical studies using EGFR targeting first generation tyrosine kinase inhibitors (TKI) erlotinib or gefitinib demonstrated that patients harboring oncogenic mutations in *EGFR* responded to these inhibitors. However, most of these trials came to the same devastating results indicating that *KRAS* mutated patients barely responded to erlotinib or gefitinib and it became literally a dogma that *KRAS* mutations mediate resistance to EGFR inhibitors.[2–4] Oncogenic KRAS was thought to be locked in a constitutive active form being independent of upstream activators, such as EGFR, thus explaining the tumor resistance to TKI. However, recent reports has challenged this view, opening the possibility to use TKI to treat *KRAS* mutated tumors.[5,6] Based in this new findings, we re-examined the use of TKI in *KRAS* mutated LUAD.

Immunohistochemical analysis of *KRAS* mutated LUAD patient biopsies showed elevated phosphorylation of various activating sites of the EGFR compared to healthy tissue, suggesting the implication of EGFR signaling in KRAS driven tumorigenesis.[7] Based on that finding, we initiated studies using genetically engineered mouse models (GEMM) of Kras mutated LUAD. In clear contrast to the clinical data, we found that genetic deletion of Egfr in $Kras^{G12D}$ driven lung tumors significantly reduced tumor growth and hence tremendously extended life of tumor bearing mice. We extended our study to human cell line derived xenografts, where we deleted EGFR in the LUAD cell line A549 using CRISPR-Cas9 technology. Indeed, EGFR deficient A549 cells exhibited significantly reduced growth *in vitro* as well as following transplantation into immunodeficient mice. Furthermore, we observed that EGFR knockout reduced the activity of mutated KRAS both in human and in mouse cell lines in agreement with previous reports.[5,6]

Based on the above, we re-evaluated the potential of EGFR targeting TKI *in vitro*. Confirming the data obtained from patients, different human and mouse LUAD cell lines harboring oncogenic *KRAS* mutations hardly responded to erlotinib or gefitinib treatment. Unexpectedly, *KRAS* mutated LUAD cell lines showed 10 – 20 fold increased sensibility towards afatinib treatment compared to erlotinib/gefitinib. Notably, afatinib is a second generation EGFR TKI which covalently binds to EGFR and also exhibits pronounced activity against all other members of the family of EGFRs, namely ERBB2, ERBB3, and ERBB4.[8] Next, we tested the potential of afatinib based therapies for *KRAS* mutated LUAD patients *in vivo* by using cell line derived as well as patient derived xenografts. In all these different models we observed a remarkable inhibition of tumor growth in response to afatinib treatment. Hence, we also examined the efficacy of EGFR TKI in already established autochthonous tumors. Therefore we started to treated mice suffering from $Kras^{G12D}$ mutated LUAD 10 weeks after tumor induction for a period of another 10 weeks. Strikingly, following afatinib administration

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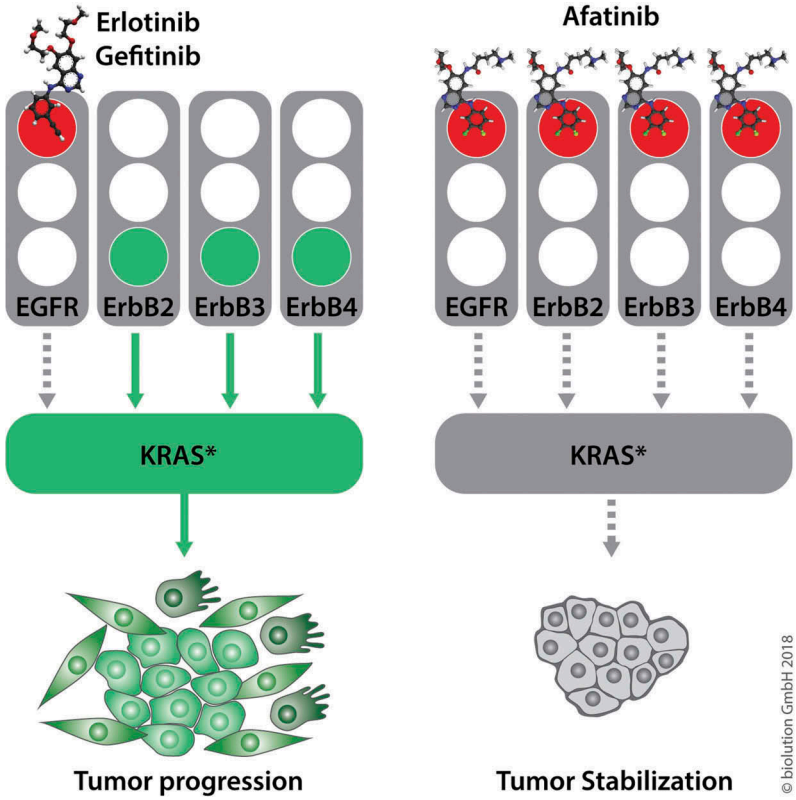

Figure 1. Afatinib restrains kirsten rat sarcoma oncogene homolog (KRAS) driven lung tumorigenesis. Afatinib, but not erlotinib neither gefitinib inhibits activation of all ERBB family members, thereby abrogating mutant KRAS activity and inhibiting tumor progression.

the tumor burden remained unchanged compared to the 10 weeks' time point (i.e. before treatment). This was in clear contrast to erlotinib or gefitinib treated mice, whose tumors remained growing comparable to the vehicle treated control mice. While the latter results confirmed the data observed in clinical trials using first generation TKI, these experiments also highlighted the clinical potential of afatinib based therapies for *KRAS* mutated LUAD patients.

Nevertheless, it remained still unclear why erlotinib or gefitinib treatment was ineffective, while the second generation TKI afatinib proved to be a promising drug to block *KRAS* driven LUAD. When culturing the EGFR deficient cells for different follow up experiments, we noticed that these cells quickly regained proliferative capacity, and after passaging these cells for up to 50 times they completely recovered from the burden of EGFR deletion. Analysis of these cells indicated increased expression and activation ERBB2 and ERBB3 most likely to compensate for the loss of EGFR. Similarly, autochthonous *Kras* driven LUAD lacking Egfr regained their proliferative capacity at late tumor stages in vivo. This was concomitant with increased ErbB2, ErbB3 and ErbB4 expression and (re)activation of downstream effectors in these tumors. Importantly, also erlotinib or gefitinib induced expression of ERBB2, 3 and 4 and activation of ERBB3 in *KRAS* mutated LUAD cells, thereby feeding the tumor escape mechanism. However, and in contrast to first generation TKIs, the pan-ERBB TKI afatinib completely blocked activation of all ERBB receptors and thereby prevented the tumor compensatory mechanism (Figure 1).[7]

While in the process of preparing our manuscript for submission, we became aware of a study conducted by Kruspig et al. In this study, the authors demonstrate increased expression of ligands of the ErbB receptors in advanced *Kras* mutated LUAD, and proof that the pan-ERBB inhibitor neratinib was highly efficient in combination with MEK inhibition.[9] Hence, their works confirms our data that *KRAS* driven LUAD are sensitive to pan-ERBB TKI. Altogether, both studies provide a solid rationale to revisit the potential of these inhibitors in clinical trials.

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