Integrated research in STAT3 in cancer stem cells. Early adaptive resistance to EGFR inhibition in EGFR mutant Non-Small-Cell-Lung-Cancer.

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There is a need to improve survival of lung cancer patients with EGFR mutations stemming from growing evidence that, following EGFR TKI inhibition, STAT3 activation occurs almost immediately, a few hours after exposure to gefitinib or erlotinib and gradually increases. The mechanisms of STAT3 activation were identified through IL-6/JAK1/STAT3 and also more recent evidence shows that erlotinib can directly induce pSTAT3 (Tyr705) by dephosphorylating PTPMeg2. Combinations of gefitinib or erlotinib with repurposed drugs such as metformin and niclosamide prevent and reverse TKI resistance in xenograft models. We were able to identify high levels of BIM mRNA expression as a predictive marker of response, PFS and OS in erlotinib-treated NSCLC patients. We posit that one third of EGFR mutant NSCLC patients expressing high BIM mRNA could be related to low SHP2 and these patients are prompted to have rapid or immediate pSTAT3 phosphorylation and gradual elevation of STAT3 at transcriptional level. EGFR mutant cell lines show low levels of SHP2 which attenuates ERK signaling and therefore preserves BIM from proteosomal degradation. At the same time, SHP2 is a negative regulator of STAT3 signaling. For more than 50% of EGFR mutant NSCLC patients with low/intermediate BIM mRNA expression we found that response rate was significantly lower (less than 40%) with shorter PFS and OS. We speculate that in this significant subgroup of patients, other RTKs can be activated such as EPHA2, AXL/MER which upregulate SHP2 and STAT3. We are investigating which of these RTKIs could be most significant to cause intrinsic resistance with low BIM mRNA expression and several synthetic lethal approaches.

The ultimate goal of such research is to have compelling evidence that single EGFR TKI therapy could be replaced by adequate combinations in two subgroups of EGFR mutant patients. Good responders with immediate STAT3 activation to be treated with EGFR TKI in combination with adequate or the most optimal repurposed drug. For patients with intrinsic resistance the aim is to identify the main target AXL/MER and/or EPHA2 and the

contribution of FGFR signaling and provide novel combinations to overcome immediate mechanisms of resistance.

Two of the main downstream effector components of EGFR, AKT and ERK are inactivated upon afatinib treatment while STAT3 is paradoxically hyperactivated via IL-6R/JAK1/STAT3[1]. Activation of nuclear factor κ-B (NFκB) could be a plausible cause for autocrine IL-6 production by afatinib. Recently, in addition to JAK1/STAT3 axis it has been shown that STAT3 is activated downstream of FGFR through PI3K. Activation of STAT3 could be even more complex and we posit that is may also involve sphingosine kinase 1 (SphK1) due to crosstalk between NFκB/IL-6 and EGFR/MAPK/SphK1.

Also, IL-6 mRNA levels are elevated about two-fold upon afatinib treatment in both EGFR mutant cell lines[1]. Inhibition of IL-6R/JAK1/STAT3 signaling pathway (by IL-6R neutralizing antibody or P6, a pan-JAK inhibitor) increases sensitivity to afatinib in H1975 and PC9-GR cells as well as in a PC9-GR xenograft model[1].

Recently, we also have noted that gefitinib only moderately attenuates AKT in the PC9 and 11-18 cell lines while STAT3 phosphorylation occurs almost immediately after two hours of exposure to gefitinib. For the first time, to the best of our knowledge, we have documented that gefitinib increased STAT3 mRNA in the PC9 cell line on days 7 and 9.

References

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