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Preview

Exploiting metabolic vulnerabilities in breast cancers with *NF1* loss

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Auf der Maur et al.[1] identify neurofibromin 1 (*NF1*) loss as a mechanism of resistance to PI3K inhibitor in breast cancer cells. *NF1* loss leads to enhanced glycolysis, which may be targeted with the antioxidant N-acetyl cysteine (NAC).

Alpelisib with fulvestrant is one of the preferred treatment options in *PIK3CA*-mutated advanced breast cancer (BC), after progression on cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) with endocrine therapy (ET). However, *PIK3CA*-mutated advanced BCs have worse outcomes compared to wildtype tumors,[2] with median progression-free survival in the range of 6 months on alpelisib with ET after prior CDK4/6i. Apart from inactivating *PTEN* mutations, adaptive rewiring, epigenetic and metabolic reprogramming in cancer cells have been implicated in resistance to PI3K inhibition.[3]

To elucidate other mechanisms of resistance to PI3K inhibition, Auf der Maur et al. performed an *in vivo* genome-wide PiggyBac transposon-mediated mutagenesis screen in parallel with an *in vivo* resistance screen in a *PIK3CA*^{H1047R}-mutated murine tumor model, and identified *NF1* as a candidate gene driving resistance to PI3K inhibition.[1] *NF1* mutations are enriched in metastatic or post-progression BC specimens (range 5%–18%), based largely on biopsies after progression on ET +/- CDK4/6i.[4,5] The role of NF1 in tumor suppression beyond regulation of the Ras-Raf-MEK and Ras-PI3K-mTOR cellular proliferation pathways[5,6] is increasingly recognised (Figure 1).

Transcriptomic analysis by Auf der Maur et al. revealed upregulation of not only MAPK activity, but also increased MYC signaling with knockout (KO) of *NF1* in the T47D cells harboring *PIK3CA* H1047R mutation.[1] Cancer cells have different metabolic features from normal cells. While both MAPK and MYC signaling are known to enhance aerobic glycolysis via the Warburg effect, and MYC with augmented oxidative phosphorylation to increase cell energy production, the impact of *NF1* loss on glycolysis and mitochondrial respiration was previously unclear. Interestingly, although *NF1* loss led to enhanced glycolysis with increased lactate production, cellular reactive oxygen species (ROS) levels were reduced with lower mitochondrial respiration, indicating less dependency on oxidative phosphorylation.[1]

Antioxidants suppress oxidation and may augment proliferation of cancer cells by reducing the generation of ROS. Conversely, antioxidants may have anticancer properties because ROS may promote tumor migration and invasion. NAC, an antioxidant, was previously shown in preclinical studies to stimulate proliferation and metastasis in melanoma and lung cancer.[9] NAC was added to alpelisib by Auf der Maur et al. with the expectation of increasing resistance to PI3K inhibition, but intriguingly NAC sensitized *NF1* KO cells to alpelisib *in vitro*, *in vivo*, and *ex vivo* in patient-derived organoids harboring *PIK3CA* H1047R mutation. No additive effect of NAC was seen in T47D control tumors *in vivo*, which remained highly sensitive to alpelisib. To evaluate the dependency of *NF1* KO cells on MYC, KJ-Pyr-9 was used to inhibit MYC pharmacologically. The increased sensitivity of *NF1* KO cells to KJ-Pyr-9 compared to control cells supported the crosstalk between *NF1* loss and MYC. However, the combination of NAC and KJ-Pyr-9 did not increase anti-proliferative effects. Only NAC, not KJ-Pyr-9, could reverse the increased glycolysis seen in *NF1* KO cells. This suggests that the increased glycolysis with *NF1* KO is independent of MYC. Notably, binimetinib (MEK inhibitor) showed only modest activity even when combined with alpelisib.

What is the mechanistic basis of NAC circumventing resistance to PI3K inhibitor with *NF1* loss? Global phosphoproteomic analysis demonstrated further reduction in mTOR signaling with addition of NAC to alpelisib in *NF1* KO cells. Given that mTOR signaling is a key driver of glycolysis, it is likely effected through mTOR inhibition. Combining everolimus (mTORC1 inhibitor) with NAC was more effective than either drug alone in diminishing cellular proliferation. Double vertical blockade of the PI3K-Akt-mTOR pathway using everolimus with alpelisib demonstrated similar effectiveness to NAC-alpelisib combination.

Is the antitumor effect of NAC restricted to (1) *NF1* loss in (2) *PIK3CA*^{H1047R}-mutated cells treated with (3) alpelisib? To address these questions, the authors silenced *NF1* in MCF7 cells that harbor *PIK3CA*^{E545K} mutation and found similar efficacy with the addition of NAC to alpelisib. Importantly, the potential activity of NAC does not appear to be unique to *NF1* loss. Similar activity was observed in T47D cells engineered to overexpress the constitutively active KRAS 4B^{G12V}

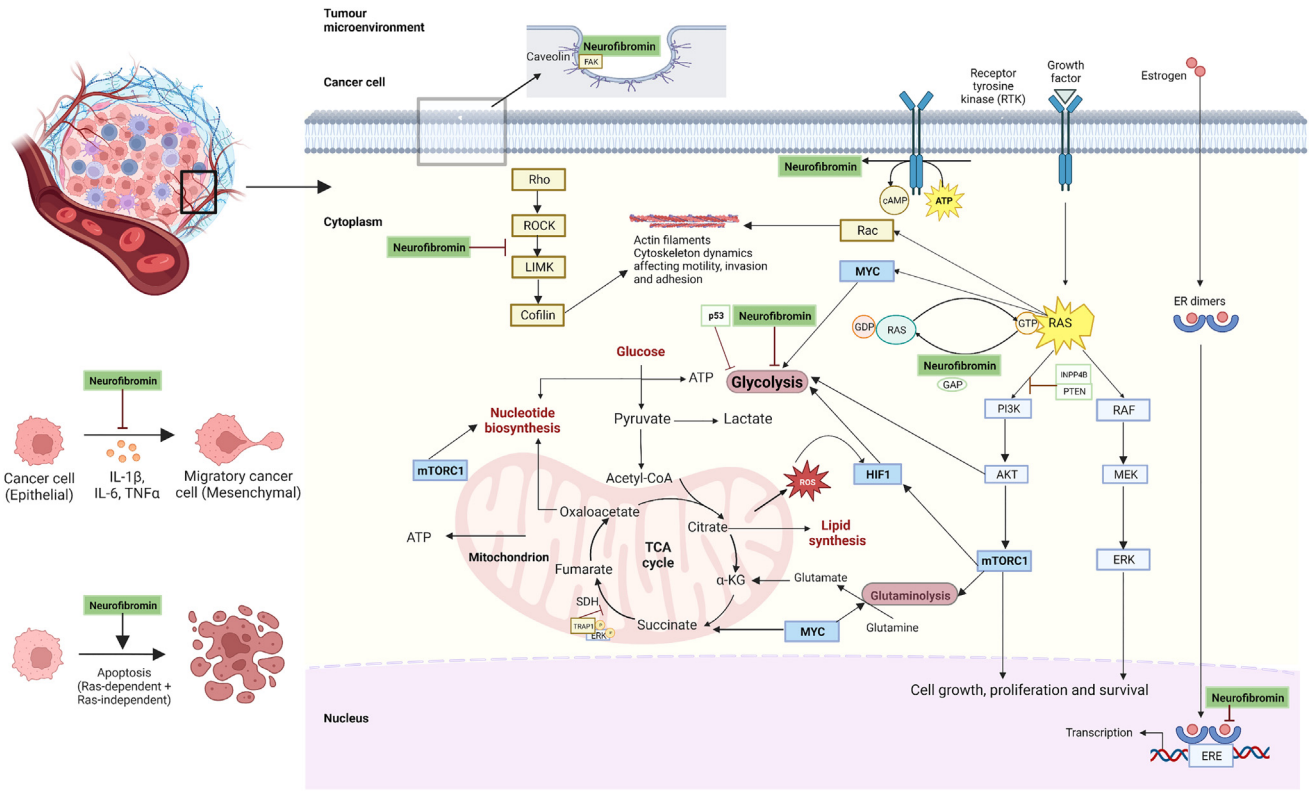

Figure 1. The roles of neurofibromin (NF1) in tumor suppression—Ras and beyond

Other roles include suppression of epithelial mesenchymal transition, promotion of apoptosis, regulation of cell adhesion and motility, positive regulation of the enzyme adenylyl cyclase (AC),[6] transcriptional co-repression of estrogen receptor (ER),[5] and regulation of cellular metabolism,[1,7] interacting with other key effectors[8] in the metabolic pathway. Figure created using BioRender.com.

mutation, supporting the efficacy of NAC co-treatment in tumors harboring other MAPK pathway alterations. Moreover, NAC co-treatment with ribociclib in *NF1* KO T47D cells overcame the resistance to CDK4/6 inhibition, while the effect with fulvestrant was marginal.

Several questions remain, including the prevalence of *NF1* alterations after exposure to PI3K inhibitors, the biological differences underlying the conflicting results with antioxidants in preclinical and clinical studies in various tumors, the complexity of redox homeostasis, and how to design new therapeutics to target these metabolic vulnerabilities in addition to repurposing old drugs. Recently, another study reported that tumor growth of neurofibromin-deficient cells was driven by decreased respiration in an ERK-dependent fashion. Tumorigenicity was hampered by NAD^+ or NAD^+ deacetylase SIRT3, with synergistic effect from ablation of TRAP1 mitochondrial chaperone.[7] In spite of such discoveries, metabolic dysregulation, one of the hallmarks of cancer, remains poorly understood. There is a pressing need for comprehensive characterization of the metabolome in human tumors beyond the glycolytic pathway. The interplay with various genomic aberrations, epigenetic remodeling, the tumor microenvironment, and the microbiome needs to be unraveled further.

In summary, NAC has promising efficacy based on this preclinical study. It is affordable and may be better tolerated than everolimus to overcome resistance to PI3K or CDK4/6 inhibition. However, the metabolic milieu and tumor microenvironment are more complex in humans than in the laboratory, with considerable heterogeneity and plasticity in response to any perturbation. The optimal dose of NAC required to revert the glycolytic phenotype is also unclear. The development of metabolic strategies to treat cancers in humans has been challenging, with disappointing results from the metformin trials,[10] though asparaginase in acute lymphoblastic leukemia is a successful example of targeting metabolic vulnerability in cancer cells.[8] To test the hypothesis generated by Auf der Maur et al., the next step would be to conduct a clinical trial in the right patients with a multi-omics approach, and translate the important findings of this study from bench to bedside.

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