INHIBITION OF P21-ACTIVATED KINASE 4 (PAK4) REVERTS IMMUNE EXCLUSION AND RESTORES ANTI-TUMOR IMMUNITY IN THE TUMOR MICROENVIRONMENT


Background P21-activated kinase 4 (PAK4) is a serine/threonine protein kinase that is mostly expressed in tumor and stroma cells. PAK4 activates tumor WNT/β-catenin pathway and regulates cellular morphology, motility, EMT, cell proliferation and survival. Recent studies also showed that PAK4 can actively exclude T cells from tumors, suggesting that therapeutic inhibition of PAK4 can increase T cell infiltration in tumor microenvironment and overcome resistance to checkpoint inhibitor immunotherapy. 1

Methods We generated PAK4 knockout (KO) clones in human and mouse tumor cells to validate its biology in vitro and in vivo. We also performed pharmacological evaluation of PAK4 inhibition using Pfizer compounds (referred to as 'PAK4i compounds' below) for their potential tumor-intrinsic and immune-regulatory roles.

Results Nanostring, qPCR and RNAseq analysis showed that PAK4 depletion led to increase of cytokine expression in tumor, including conventional dendritic cell (cDC)- recruiting chemokine CCL4, and type I IFN / ISG pathway genes that are associated with MHC upregulation such as CXCL10. In addition, PAK4 KO sensitizes B16F10 tumors to anti-PD-1 treatment and increases infiltration of cDC and T cells in the tumor microenvironment. We also showed that small molecule PAK4i compounds induced more potent cancer cell growth inhibition over treated normal PBMCs. PAK4i compounds also increased immune-activating and decreased immune exclusion genes in B16F10 cells and tumor explants in vitro. Although PAK4 target engagement is demonstrated by CETSA assay, the compound potency on modulating PAK4 downstream Wnt/β-catenin pathway is low, suggesting that the aforementioned phenotypic changes induced by PAK4i compounds may be partially attributed to other off-target effects.

Conclusions Collectively, our data suggests that genetic depletion or pharmacological inhibition of PAK4 may induce immune-activating cytokine production in tumor cells, revert immune cell exclusion in tumor microenvironment, and synergize with checkpoint blockade therapies. However, further optimization on these PAK4i compounds is needed to improve its specificity on modulating PAK4 enzyme activities.

REFERENCE

Ethics Approval All animal studies were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of Pfizer. Approved protocol # LAJ-2019-01347

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