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Allosteric ClpP agonist ONC206 alters mitochondrial metabolism, stress response and chromatin accessibility to elicit apoptosis in pheochromocytoma

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BACKGROUND

Imipridone ONC206, a derivative of dordaviprone (ONC201), is a caseinolytic protease P (ClpP) agonist and dopamine receptor D2/3 (DRD2/3) antagonist with nanomolar potency. Dordaviprone demonstrated responses in patients with neuroendocrine pheochromocytoma-paraganglioma (PC-PG) including tumors with SDHB/FH mutations. We characterized the mechanism of action, in vitro efficacy relative to standard of care (SOC) and dordaviprone, response determinants and acquired resistance for ONC206 in PC models.

METHODS

ClpP was expressed in E. coli and purified by His tag affinity for biochemical assays. PC (hPheo1, PC12, MPC10) and fibroblast (HFF-1, MRC-5) cells were commercially sourced. Cell viability (CellTiter-Glo, CyQuant), apoptosis (Caspase Glo, annexin V) assays, seahorse analysis and multi-omics were conducted in PC cells treated with vehicle/ONC206. Resistant hPheo1 cells were generated by passaging with increasing ONC206 concentrations.

RESULTS

Co-crystallization with ClpP revealed an allosteric ligand interaction with distinctions in the ONC206-ClpP resolved crystal structure relative to the dordaviprone-bound or apo complexes. ONC206 was a more potent agonist than dordaviprone in cell-free human ClpP casein/peptide assays. Adrenal gland tumors emerged as most sensitive when ONC206 cytotoxicity was assessed in a panel of 432 human cancer cell lines. Accordingly, PC lines exhibited increased nanomolar sensitivity to ONC206 (~6 fold) relative to dordaviprone in cell viability assays. ONC206 induced dose- and time-dependent apoptosis in PC but not fibroblast cells. ONC206 demonstrated superior cell viability inhibition and/or apoptosis induction in PC lines relative to dordaviprone, temozolomide, sunitinib and belzutifan at equivalent and/or therapeutically relevant concentrations. In hPheo1 cells, CRISPR-mediated SDHB or FH knockout and DRD2 overexpression did not impact ONC206 sensitivity while ClpP knockout impaired ONC206 sensitivity. Proteomics indicated inhibition of mitochondrial metabolism by ONC206, including OXPHOS/TCA cycle while metabolomics revealed elevated α-ketoglutaric acid, 2-hydroxyglutaric acid and reduced succinic acid, fumaric acid in a ClpP-dependent manner. Consistent with epigenetic regulation by metabolites, ATACseq revealed ONC206 altered chromatin accessibility while RNAseq demonstrated upregulated stress response, apoptosis and downregulated metabolism-related pathways. Western blot confirmed ONC206 downregulated mitochondrial proteins, neuroendocrine markers and upregulated stress response. Whole exome

sequencing of acquired resistant cells revealed diverse ClpP missense and/or termination mutations, further confirmed using PCR and/or western blot. Seahorse analysis showed ONC206 inhibited mitochondrially-derived ATP in parental but not acquired resistant lines. Overexpression of wild-type ClpP restored ONC206 sensitivity in acquired resistant lines.

CONCLUSIONS

ONC206 is a potent novel agent that is superior to SOC and dordaviprone in PC. ClpP mediates response and acquired resistance to ONC206 in PC.

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