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BH3-mimetics synergise with systemic therapies to induce apoptosis in patient-derived neuroendocrine tumour organoids

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BACKGROUND

Neuroendocrine tumours (NETs) are growing rapidly in prevalence, yet current systemic treatments result predominantly in prolonged disease stabilisation, necessitating novel approaches to induce objective tumour regression and prevent recalcitrant disease. Single-cell transcriptomic profiling of NET patients demonstrated expression of the BCL2 family of apoptosis inhibitors—comprising BCL-2, BCL-w, BCL-xL, and MCL-1—which may contribute to cancer cell persistence following treatment. We hypothesised that BH3-mimetics, drugs targeting the BCL2 family, may induce cell death in NETs and represent an effective therapy, either alone or in combination with existing standard-of-care (SOC).

METHODS

NET patient-derived organoids (PDOs) were established from fresh, surplus surgical specimens, cultured for 3–4 weeks in hydrogel-coated 384-well plates, and treated with combinations of BH3-mimetics and SOC agents. Treatment response was assessed by confocal imaging using fluorescent, non-toxic dyes to measure metabolic activity and Annexin V to detect apoptosis. Further characterisation by Western blotting and RNA-seq were also conducted to validate the pro-apoptotic mechanisms of BH3-mimetics in NET cells.

RESULTS

We have established PDOs from primary (small intestine, pancreas, colon) and metastatic (lymph node, liver) low-grade, well-differentiated NETs with >90% success, and validated their expression of neuroendocrine markers and key apoptosis regulators at both RNA and protein levels. Inhibition of all anti-apoptotic BCL2 proteins with a combination of BH3-mimetics resulted in prominent cell death alongside upregulation of apoptosis markers Annexin V, cleaved Caspase-3, and cleaved PARP, indicating an active apoptotic machinery in NETs. The drug-specific modulation of different BCL2 proteins was also confirmed, suggesting that the observed cell death is an on-target effect of BH3-mimetics. Next, treatment with BH3-mimetics specific to single BCL2 proteins identified MCL-1 to be essential, but not sufficient, for NET survival. Finally, we found Pelcitoclax—a well-tolerated BH3-mimetic in clinical studies—to effectively induce cell death when combined with current NET SOCs, while the single treatments exhibited a significantly lower effect. A strong synergistic effect was observed when Pelcitoclax was combined with Cabozantinib or Sunitinib, and to a lesser extent with Everolimus, while no synergy was observed with Octreotide.

CONCLUSIONS

This study demonstrates that the apoptotic machinery of well-differentiated neuroendocrine tumours is active and that their survival depends on the activity of BCL2 family proteins. Inhibition of these proteins by BH3-mimetics combined with SOC effectively induces cell death in patient-derived preclinical models. Overall, our data warrant investigating BH3-mimetics with SOC in patients with NETs as a novel therapeutic approach.

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