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Combination of angiogenesis and HIF-2α blockade: synergistic pair worth exploring in neuroendocrine tumors

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BACKGROUND

Inhibition of angiogenesis via vascular endothelial growth factor receptor (VEGFR) blockade has revealed therapeutic efficacy in advanced neuroendocrine tumors (NETs), most recently with Cabozantinib in the CABINET trial. Belzutifan, a hypoxia-inducible factor (HIF)-2α inhibitor, has demonstrated activity in Von Hippel-Lindau (VHL)-associated pancreatic NETs (pNETs). Evidence has also shown that VHL gene impairment by promoter methylation and deletion occurs in nearly 25% of sporadic pNETs. The HIF pathway regulates several oncogenes related to proliferation, angiogenesis, invasion, and metastasis including VEGF. Interaction between angiogenesis and the hypoxia signaling pathway presents an opportunity to repress VEGF at both the transcription and receptor level.

METHODS

BON and QGP (human pNET) and STC-1 (mouse small bowel NET) cell lines were used. For hypoxia experiments, cells were maintained in a hypoxic incubator set to 3.0% O_2 and 5.5% CO_2. Cells were treated with serial dilutions of Belzutifan and Cabozantinib ranging from 250 μM to 7.8 μM and 100 μM to 3.1 μM, respectively. Drug interaction and synergy were analyzed using the SynergyFinder Plus platform. The Zero Interaction Potency (ZIP) synergy score was calculated for each cell line at varying drug concentrations under both normoxic and hypoxic conditions. Cell viability was measured using MTT and trypan blue exclusion assays after 48 hours. DMSO and untreated cells served as controls. Additionally, spheroids from each cell line were treated with Belzutifan, Cabozantinib, and their combination and placed in hypoxia for 48 hours. HIF-2α expression levels were analyzed via Western blot.

RESULTS

The combination of Belzutifan and Cabozantinib demonstrated varying synergy across BON, QGP, and STC-1 cell lines under normoxic and hypoxic conditions. In BON cells, moderate synergy was observed in hypoxia (ZIP: 34.74) and normoxia (ZIP: 27.26), with cell viability significantly reduced under hypoxia (50.10% at the highest concentration). Strong drug synergy was observed in QGP cells under both hypoxia (ZIP: 168.01) and normoxia (ZIP: 131.03). STC-1 cells showed moderate synergy under hypoxia (ZIP: 36.89), but minimal synergy under normoxia (ZIP: 0.90). Western blot analysis revealed that HIF-2α expression decreased as drug concentrations increased in hypoxic conditions. Cell viability and spheroid drug synergy studies confirmed that combination treatment led to highest cell death in hypoxia.

CONCLUSIONS

The combination of Belzutifan and Cabozantinib demonstrates strong synergy in QGP cells and moderate synergy in BON and STC-1 cells. The reduction in HIF-2α expression and increased cell death with combination treatment in hypoxia support further exploration of this treatment.

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