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Phosphoproteomic Mass Spectrometry Reveals A Novel Therapeutic Target in Well-Differentiated Gastroenteropancreatic Neuroendocrine Tumors

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BACKGROUND

Though surgical debulking is an accepted therapeutic strategy for gastroenteropancreatic neuroendocrine tumors (GEP NETs), most patients will develop liver recurrence limiting overall survival, highlighting need for additional therapy. Given limited utility of mutational analysis to identify new drug targets, we sought to employ novel techniques to unveil tumor signaling pathways and kinases of interest.

METHODS

Phospho- and total proteomic analysis was performed on snap-frozen small bowel NET (SBNET) and pancreatic NET (PNET) liver metastases and adjacent normal liver. Identified phosphorylation sites were back-mapped onto upstream kinases using the *Kinase Library*, a computational motif dataset of the entire human serine/threonine kinome, and used to nominate kinases driving these tumors based on statistically significant enriched substrates in the mass-spec dataset.

Small molecule inhibitors were selected based on candidate kinase targets, and patient-derived organoids (PDOs) were generated for pharmacologic testing. PDOs were treated for 96-hours, with drug response measured by CellTiter-Glo and fluorescent imaging.

RESULTS

Liver metastases and adjacent liver parenchyma were obtained from 9 SBNET patients (Grade I n=6, Grade II n=3) and 4 PNET patients (Grade I n=1, Grade II n=3), with identification of ~19,000 peptides and 2,176 independent phosphopeptides. Number and distribution of total peptides/proteins detected by mass spectrometry were similar between metastatic NETs and hepatic parenchyma. Mean abundance of phosphopeptides in NET samples increased by over 2-fold. After computational analysis of tumor-enriched phosphosites, several kinase signatures emerged including regulators of MAPK signaling, protein secretion, and activation of a casein kinase (CK) 1 isoform. We selected CX-4945 (silmitasertib), a casein-kinase 2 inhibitor, for PDO validation, as CK1 has no available clinically relevant inhibitors.

PDOs were derived from 13 unique PNET metastases from 3 patients and 8 unique SBNET metastases from 3 patients. Establishment rate was 84.6% (11/13) for PNET and 75% (6/8) for SBNET.

IHC demonstrated GEP-NET marker expression in PDOs. Silmitasertib exhibited an IC_{50} 0.18-0.75 μM in SBNETs and IC_{50} 0.79-4.75 μM in PNETS.

CONCLUSIONS

Phospho-MS reveals novel signal dysregulation in well-differentiated GEP-NET liver metastases. Our data suggests that CK-2 inhibition has potential efficacy for Grade I and Grade II SBNETs and PNETs.

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