A Patient-derived Xenograft Model for Pancreatic Neuroendocrine Tumors

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Purpose

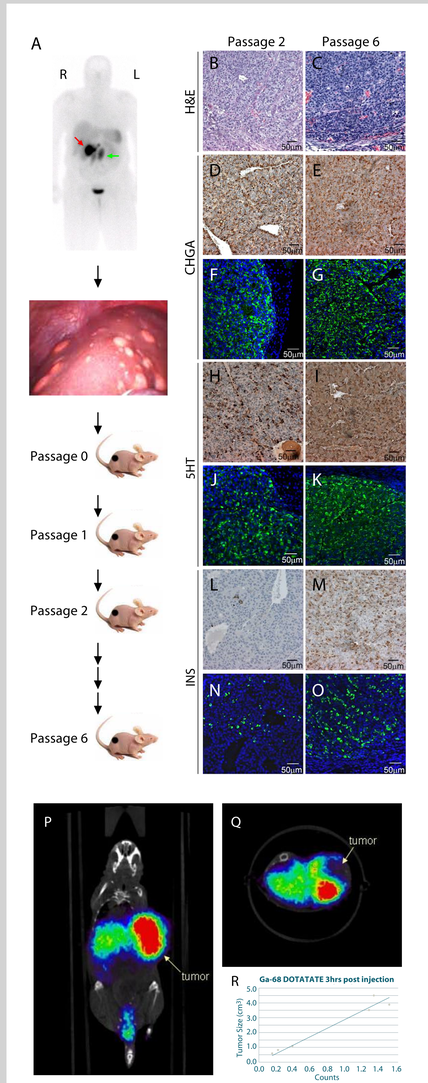
Patients with pancreatic neuroendocrine tumors (PNETs) commonly have extensive metastatic disease and therefore require a drug therapy. However, treatment options remain limited as there is a lack of experimental models that reliably reflect PNET disease. We therefore developed a patient-derived xenograft model of PNET (PDX-PNET), and then evaluated two mTOR inhibitor drugs in this model: FDA-approved everolimus and the investigational new drug sapanisertib.

Experimental Design

A patient with PNET liver metastases producing insulin underwent surgery to ameliorate refractory hypoglycemia. PNET tissue was implanted subcutaneously into nude mice, successfully passaged for several generations, and then histologically and genetically characterized. Mice bearing PNET xenografts were treated with everolimus or sapanisertib.

Results

1 Generation and characterization of the patient-derived xenograft model of PNET (PDX-PNET).

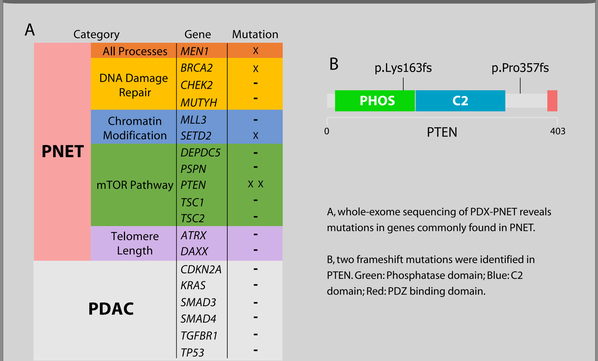


A, non-diagnostic portions of metastasized PNETs were removed during the hepatic resection and expanded in a cohort of female athymic nude mice.

B-O, confirmation of PDX-PNET histology and phenotype. H&E staining (B and C); immunohistochemistry and immunofluorescent analysis of select neuroendocrine biomarkers: chromogranin A (CHGA, D-G), serotonin (5-HT, H-K) and insulin (INS, L-O).

P-R, Gallium-68 DOTATATE PET-CT imaging of the PDX-PNET model. P, coronal view. Q, axial view. The tumor appears orange in the coronal and axial views. R, relationship between tumor size and gallium-68 DOTATATE PET-CT detection.

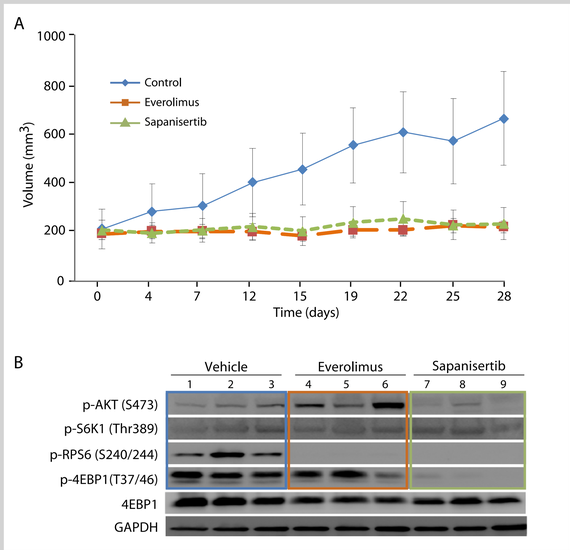
2 PDX-PNETs harbor mutations in genes commonly associated with PNETs and mTOR pathway activation.



A, whole-exome sequencing of PDX-PNET reveals mutations in genes commonly found in PNET.

B, two frameshift mutations were identified in PTEN. Green: Phosphatase domain; Blue: C2 domain; Red: PDZ binding domain.

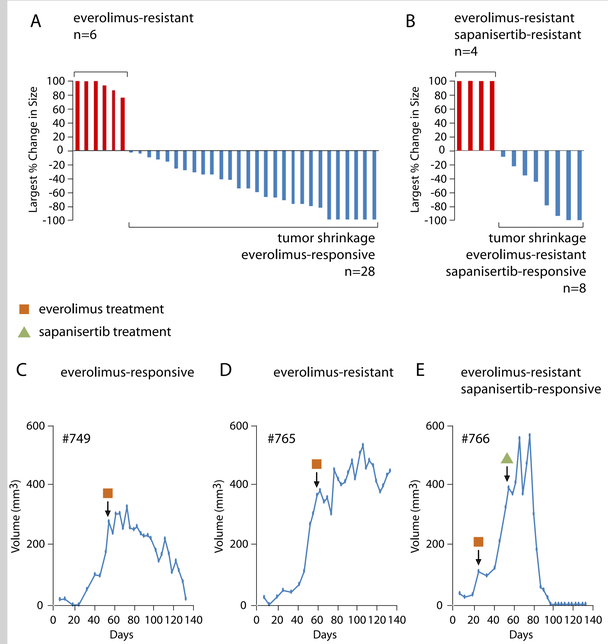
3 Response of PDX-PNETs to mTOR inhibitor drugs everolimus and sapanisertib.



A, growth chart of PDX-PNETs treated with vehicle control, everolimus or sapanisertib for 28 days (vehicle control n=5, everolimus n=6, sapanisertib n=6). Data are statistically significant between vehicle control and everolimus treated animals on days 19, 22 and 28, and between vehicle control and sapanisertib treated animals on day 28 (Student's t-test, *p<0.05).

B, Western blot analysis of mTOR pathway targets in PDX-PNETs harvested after 28 days of treatment with either vehicle control, everolimus, or sapanisertib. Each lane represents a single xenograft harvested from a unique mouse. Note the reduction in both mTORC1 targets (4EBP1 and RPS6), and the mTORC2 target AKT with sapanisertib treatment.

4 Sapanisertib causes tumor shrinkage in most everolimus-resistant PDX-PNETs.

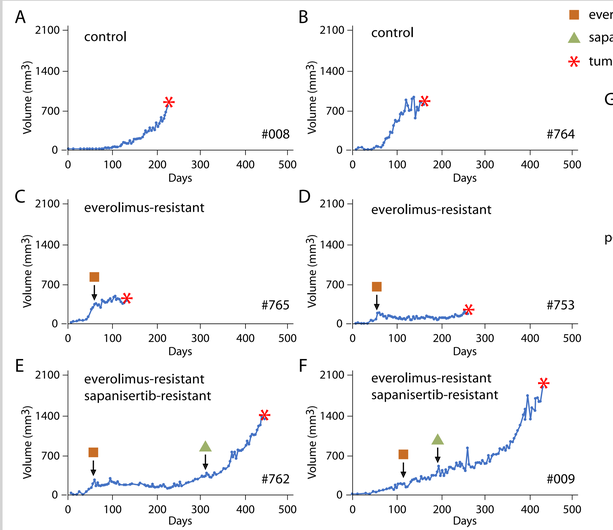


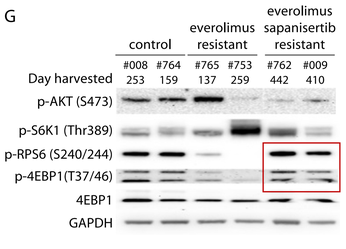
A, waterfall plot of the largest percentage change from baseline in size of PDX-PNETs treated with everolimus. Note that 18% (6/34) are everolimus-resistant.

B, waterfall plot of the largest percentage change from baseline in size of everolimus-resistant PDX-PNETs treated with sapanisertib. Note that 67% (8/12) were responsive to sapanisertib.

C-E, growth chart examples of everolimus-responsive PDX-PNET (C), everolimus-resistant PDX-PNET (D), and everolimus-resistant, sapanisertib-responsive PDX-PNET (E).

5 Everolimus-sapanisertib double-resistant PDX-PNETs maintain mTOR pathway activation.





A-F, growth chart examples of vehicle control (A, B), everolimus-resistant (C, D), and everolimus-sapanisertib double-resistant PDX-PNETs (E, F).

G, Western blot analysis of PDX-PNETs harvested from (A-F). Note that mTORC1 targets 4EBP1 and RPS6 are highly phosphorylated in the everolimus-sapanisertib double resistant PDX-PNETs (red box) while phosphorylation of

Conclusions

The PDX-PNET model is the first valid and available PDX model for PNET, and predicts that sapanisertib may serve as an effective new treatment option for patients with PNET or everolimus-resistant PNET.

Support

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