Establishment of a Patient-derived Xenograft Model of Pancreatic Grade 3 Well-differentiated Neuroendocrine Tumor

Yosuke Kasai[1, 2], Grace E. Kim[2, 3], Byron Hann[2], David B. Donner[1, 2], Robert S. Warren[1, 2], Emily K. Bergsland[2, 4], Eric K. Nakakura[1, 2]

Department of [1]Surgery, [3]Pathology, and [4]Medicine, University of California, San Francisco, [2]UCSF Helen Diller Family Comprehensive Cancer Center

GO BACK TO KIOSK MENU

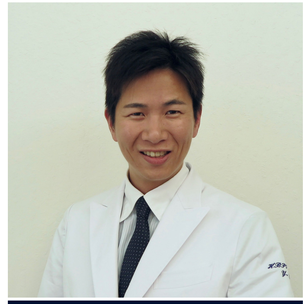
Abstract

Background: Recently, a new pathologic category of pancreatic neuroendocrine tumors (PNETs), grade 3 well-differentiated-PNET (G3 WD-PNET), was recognized by the WHO with an intermediate prognosis compared to G2 WD-PNET and G3 poorly differentiated neuroendocrine carcinoma. The molecular and clinical features of G3 WD-PNET are ill-defined, and no standard treatment exists. We developed a patient-derived xenograft (PDX) model of G3 WD-PNET as a platform to study its biology and to inform prospective trials.

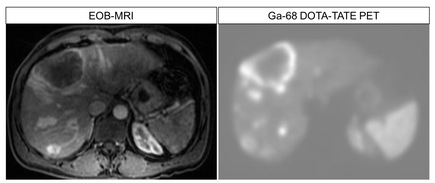
Methods: Surgically resected liver metastasis tissue from a patient with a G3 WD-PNET was implanted s.c. into NSG mice. Pathologic examination of the original patient tumor revealed a WD-PNET with 33 mitotic figures/10 hpf, Ki67 index 47%, and loss of ATRX, indicating a G3 WD-PNET. PDX tumors were passed into the next generation when the tumor volume reached 1,000-2,000 mm^3. The morphologic and molecular features of the PDX tumors were compared to the original tumor. Mice bearing PDX tumors were treated with control vehicle or everolimus (5 mg/kg/day p.o.) for 28 days.

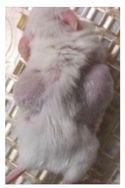
Results: Tumors grew successfully in all mice (100% take rate). Chromogranin A was diffusely positive, and the mitotic figures and Ki67 index were comparable with those of the original patient tumor. The patient and PDX tumor showed similar phospho-kinase activity, including phosphorylation of mTOR and p53. These findings were consistent with targeted sequencing results of the patient tumor showing mutations in p53 and TSC2, a negative regulator of mTOR. Everolimus significantly inhibited G3 WD-PNET PDX growth compared to control vehicle (median volume change -48% versus 150%, Wilcoxon $P = 0.005$), showing the importance of the mTOR pathway for tumor growth.

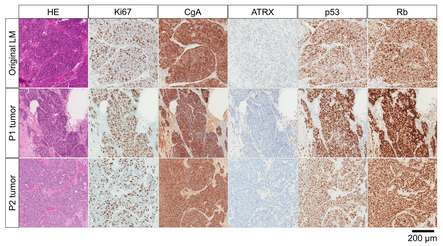
Conclusion: We successfully established a G3 WD-PNET PDX model. This model will facilitate studies of the underlying biology of G3 WD-PNET and help inform prospective clinical trials, which are desperately needed for this new disease.

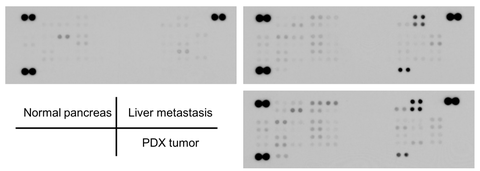


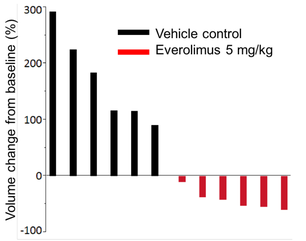
Yosuke Kasai, MD, PhD

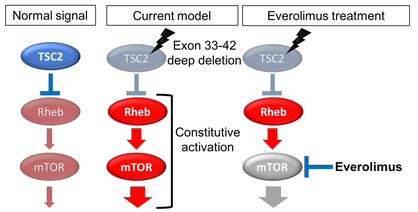












Establishment of a Patient-derived Xenograft Model of Pancreatic Grade 3 Well-differentiated Neuroendocrine Tumor

Yosuke Kasai[1, 2], Grace E. Kim[2, 3], Byron Hann[2], David B. Donner[1, 2], Robert S. Warren[1, 2], Emily K. Bergsland[2, 4], Eric K. Nakakura[1, 2]
Department of [1]Surgery, [3]Pathology, and [4]Medicine, University of California, San Francisco, [2]UCSF Helen Diller Family Comprehensive Cancer Center

GO BACK TO KIOSK MENU

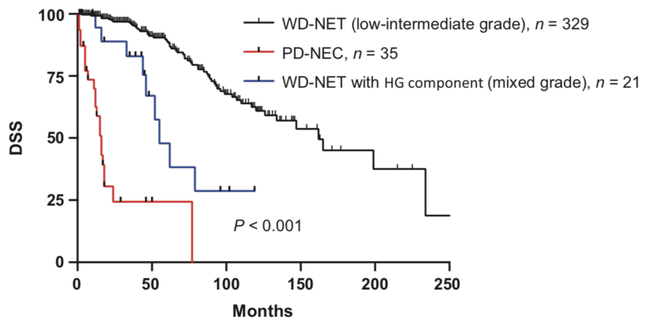
Introduction

WHO2010 classification

Classification/Grade		Ki67 index	MI /10HPF
PNET	G1	≤ 2%	< 2
	G2	3-20%	2-20
PNEC	G3 Small cell Large cell	> 20%	> 20

WHO2017 classification

Differentiation	Classification /Grade	Ki67 index	MI /10HPF
Well-differentiated	PNET G1	< 3%	< 2
	PNET G2	3-20%	2-20
	PNET G3	**> 20%**	**> 20**
Poorly-differentiated	PNEC G3 Small cell Large cell	> 20%	> 20



Tang LH, et al. Clin Cancer Res. 2016

G3 WD-PNET

Molecular features ?

- ✓ Genetics/Epigenetics
- ✓ Signaling pathways

Diagnosis ?

- ✓ Morphology
- ✓ Biomarkers (DAXX, ATRX, p53, Rb, etc.)

Treatment ?

- ✓ Cytotoxic chemotherapy
- ✓ Molecular targeted therapy
- ✓ SSA
- ✓ PRRT

Objective

1) Establish a PDX model of G3 WD-PNET

2) Investigate the biology and identify new treatments for G3 WD-PNET with this model

Establishment of a Patient-derived Xenograft Model of Pancreatic Grade 3 Well-differentiated Neuroendocrine Tumor

Yosuke Kasai[1, 2], Grace E. Kim[2, 3], Byron Hann[2], David B. Donner[1, 2], Robert S. Warren[1, 2], Emily K. Bergsland[2, 4], Eric K. Nakakura[1, 2]
Department of [1]Surgery, [3]Pathology, and [4]Medicine, University of California, San Francisco, [2]UCSF Helen Diller Family Comprehensive Cancer Center

GO BACK TO KIOSK MENU

Methods

Patient: 47 y.o. man

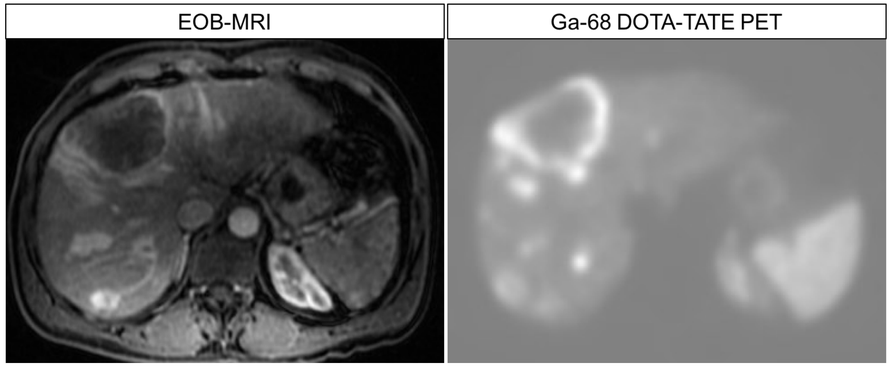
Diagnosis:
- ✓ Pancreatic tail neuroendocrine tumor (6.4 cm)
- ✓ Multiple liver metastases (the largest tumor 6.9 cm in S4)
- ✓ Bone metastasis (Th9)

Hormone secretion: None

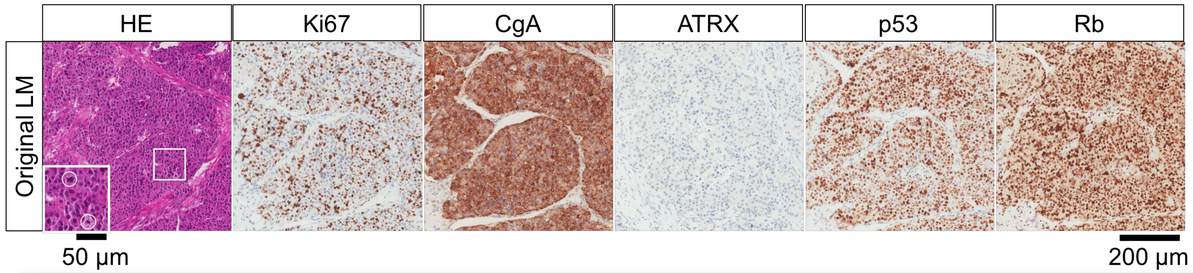
Preoperative treatment:
- ✓ Radiation for Th9
- ✓ Capecitabine/Temzolamide (6 cycles)

Surgical procedure:
- ✓ Distal pancreatectomy
- ✓ Debulking surgery of liver metastases (including S4 tumor)

Preoperative imaging of the S4 liver metastasis



Pathological findings of S4 liver metastasis: Well-differentiated morphology with 33 mitoses/10 HPFs (white circles indicate mitosis). Immunohistochemical stain result in tumor cells: Ki67 index of 47%, diffuse positive chromogranin A (CgA), loss of ATRX, strong nuclear staining of p53, and intact nuclear staining of Rb.



Targeted gene sequencing of the primary PNET

Gene	Variant
CDKN1B	Deep deletion
MEN1	p.W441fs
TP53	p.G266V
ATM	p.E2272K
TSC2	Exon 33-42 deep deletion

The fragmented tissue of the S4 liver metastasis was immediately implanted subcutaneously into male NSG mice (P0). PDX tumors were passed into the next generation when the tumor volume reached 1,000-2,000 mm^3.

Establishment of a Patient-derived Xenograft Model of Pancreatic Grade 3 Well-differentiated Neuroendocrine Tumor

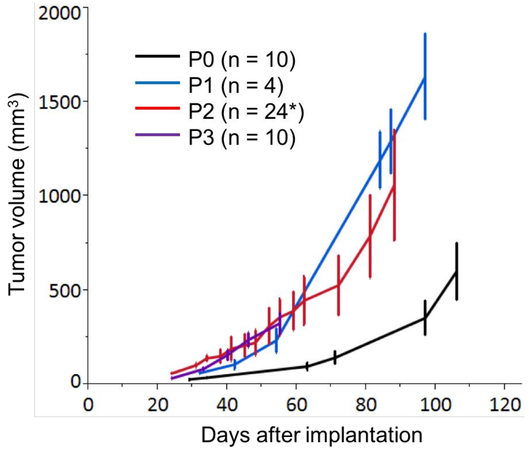
Yosuke Kasai[1, 2], Grace E. Kim[2, 3], Byron Hann[2], David B. Donner[1, 2], Robert S. Warren[1, 2], Emily K. Bergsland[2, 4], Eric K. Nakakura[1, 2]
Department of [1]Surgery, [3]Pathology, and [4]Medicine, University of California, San Francisco, [2]UCSF Helen Diller Family Comprehensive Cancer Center

GO BACK TO KIOSK MENU

Result 1. PDX tumors exhibit similar molecular features as the original tumor

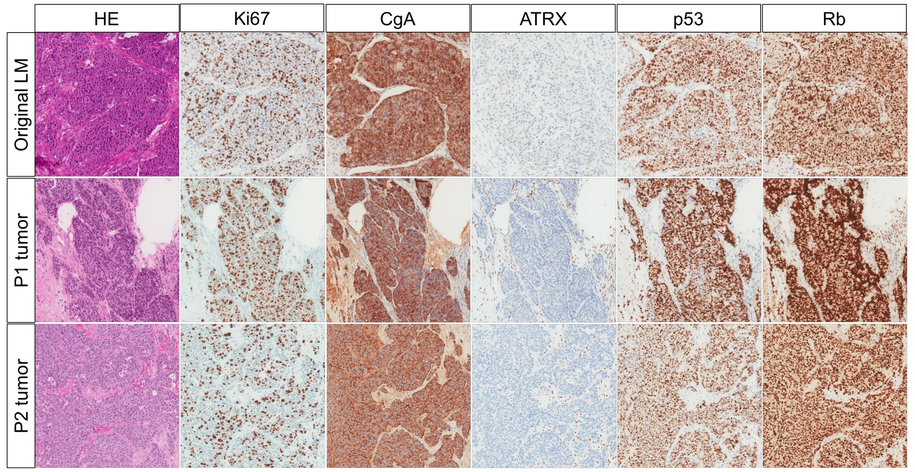
Fig. 1. (A) All implanted tumors successfully grew as of P3 (100% take rate). **(B)** PDX tumors exhibited similar morphology, proliferation, and staining patterns of CgA, ATRX, p53, and Rb as the original liver metastasis. **(C, D)** Phospho-kinase array showed a similar active kinase profile of the PDX tumor as the original liver metastasis, including enhanced phosphorylation of mTOR and p53 (S392 and S46) and attenuated phosphorylation of p27 (encoded by *CDKN1B* gene).

A



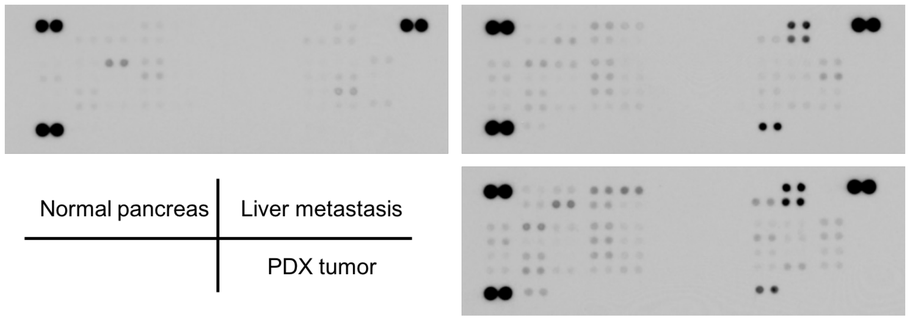
*20 of 24 P2 tumors were used for drug sensitivity test after day 34, and were excluded from the growth curve thereafter.

B



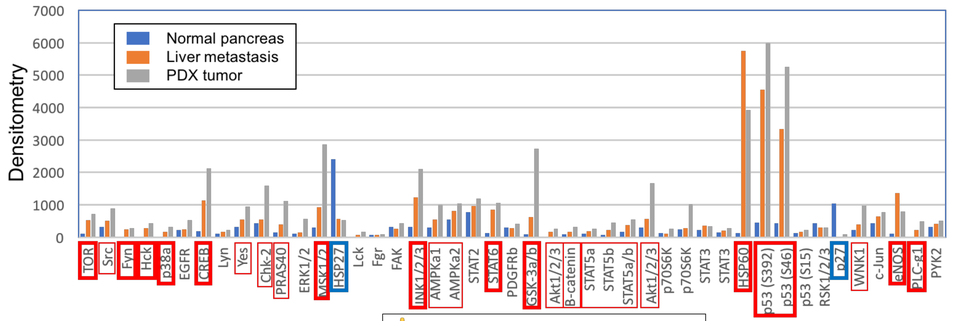
CLICK TO ENLARGE

C



CLICK TO ENLARGE

D



CLICK TO ENLARGE

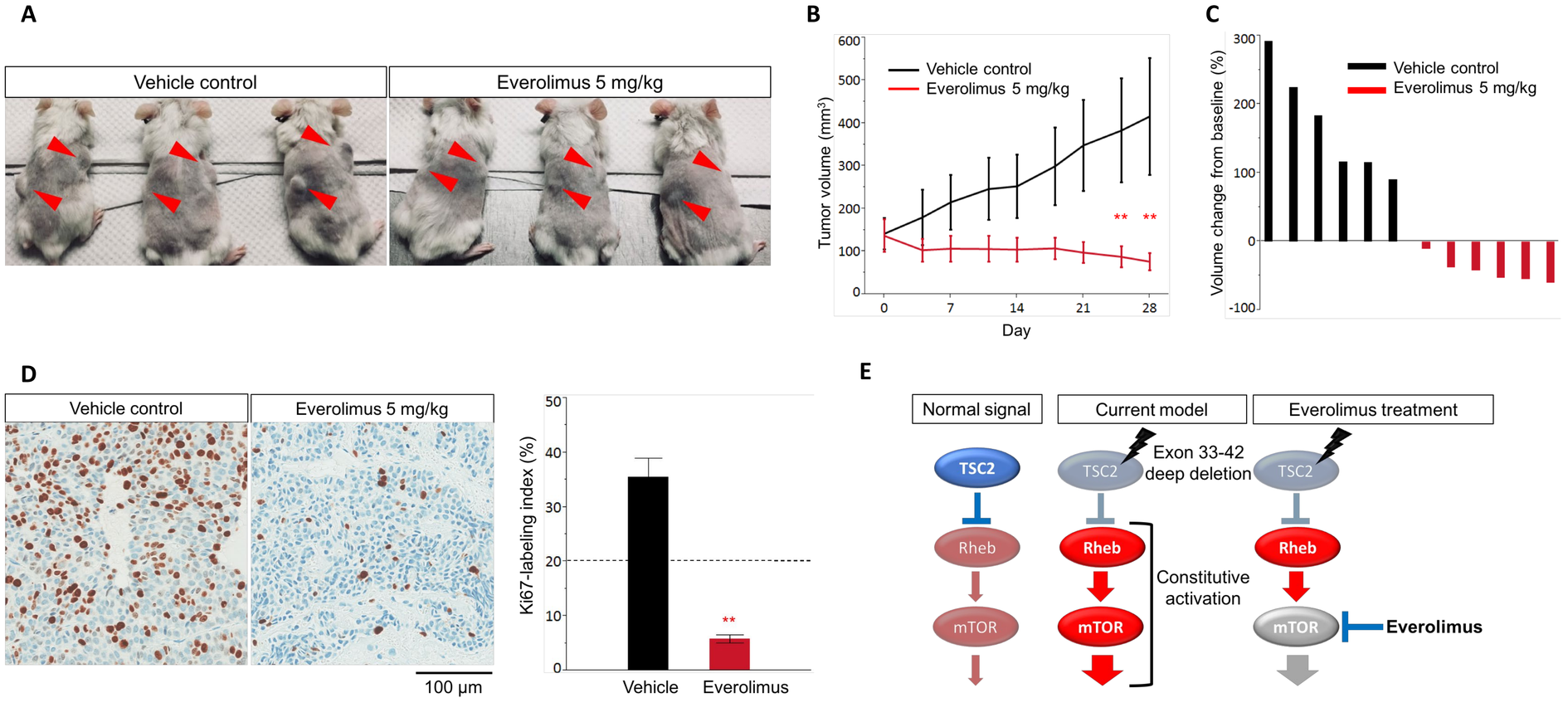
Establishment of a Patient-derived Xenograft Model of Pancreatic Grade 3 Well-differentiated Neuroendocrine Tumor

Yosuke Kasai[1, 2], Grace E. Kim[2, 3], Byron Hann[2], David B. Donner[1, 2], Robert S. Warren[1, 2], Emily K. Bergsland[2, 4], Eric K. Nakakura[1, 2]
Department of [1]Surgery, [3]Pathology, and [4]Medicine, University of California, San Francisco, [2]UCSF Helen Diller Family Comprehensive Cancer Center

GO BACK TO KIOSK MENU

Result 2. Everolimus suppressed PDX tumor growth.

Fig. 2. PDX tumor-bearing mice were treated once daily with everolimus (5 mg/kg) or vehicle p.o. for 28 days. **(A)** Gross appearance of PDX tumors 28 days after treatment initiation. **(B)** Growth curves of the 2 groups. **(C)** Waterfall plots of each tumor volume on day 28 compared to that on day 0. The median volume changes were 150% (vehicle) and -48% (everolimus), Wilcoxon $P < 0.05$. **(D)** Ki67 positive proliferating tumor cells significantly decreased in the PDX tumors treated by everolimus. **(E)** A possible mechanism for the activation of mTOR signaling and the effect of everolimus in the current PDX model. Data were expressed as the mean and the standard error, and were compared using the Wilcoxon's test. ** $P < 0.05$.



Establishment of a Patient-derived Xenograft Model of Pancreatic Grade 3 Well-differentiated Neuroendocrine Tumor

Yosuke Kasai[1, 2], Grace E. Kim[2, 3], Byron Hann[2], David B. Donner[1, 2], Robert S. Warren[1, 2], Emily K. Bergsland[2, 4], Eric K. Nakakura[1, 2]
Department of [1]Surgery, [3]Pathology, and [4]Medicine, University of California, San Francisco, [2]UCSF Helen Diller Family Comprehensive Cancer Center

GO BACK TO KIOSK MENU

Conclusion

- ✓ We successfully established a G3 WD-PNET PDX model, which faithfully retains the molecular features of the original patient tumor.
- ✓ This model will facilitate studies of the underlying biology of G3 WD-PNET and help inform prospective clinical trials.

References

1. Lloyd RV, Osamura RY, Kloppel G, Rosai J. WHO classification of tumours of endocrine organs, WHO/IARC classification of tumours, 4th edition, volume 10. Lyon: International Agent for Research on Cancer, World Health Organization, 2017.
2. Tang LH, Untch BR, Reidy DL, et al. Well-Differentiated Neuroendocrine Tumors with a Morphologically Apparent High-Grade Component: A Pathway Distinct from Poorly Differentiated Neuroendocrine Carcinomas. Clin Cancer Res. 2016 Feb;22: 1011-7.
3. Tang LH, Basturk O, Sue JJ, Klimstra DS. A Practical Approach to the Classification of WHO Grade 3 (G3) Well-differentiated Neuroendocrine Tumor (WD-NET) and Poorly Differentiated Neuroendocrine Carcinoma (PD-NEC) of the Pancreas. Am J Surg Pathol. 2016 Sep;40: 1192-202.
4. Raj N, Valentino E, Capanu M, et al. Treatment Response and Outcomes of Grade 3 Pancreatic Neuroendocrine Neoplasms Based on Morphology: Well Differentiated Versus Poorly Differentiated. Pancreas. 2017 03;46: 296-301.
5. Mizuno Y, Kudo A, Akashi T, et al. Sunitinib shrinks NET-G3 pancreatic neuroendocrine neoplasms. J Cancer Res Clin Oncol. 2018 Jun;144: 1155-63.
6. Gao H, Korn JM, Ferretti S, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015 Nov;21: 1318-25.
7. Scarpa A, Chang DK, Nones K, et al. Whole-genome landscape of pancreatic neuroendocrine tumours. Nature. 2017 03;543: 65-71.

Acknowledgement

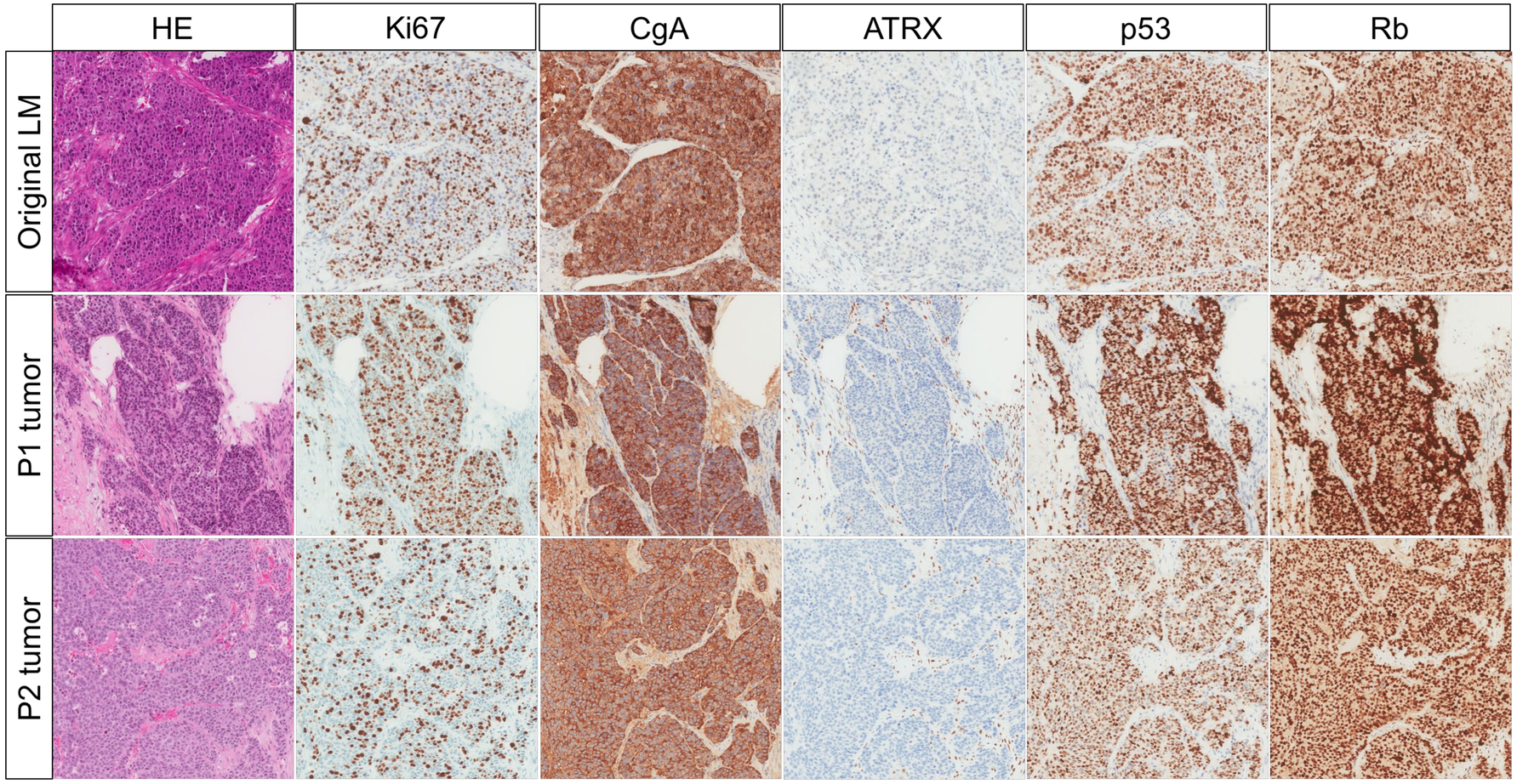
This study was supported by NETRF and UCSF Distinguished Professorship in Anatomic Pathology Endowment held by Dr. Kim.

Establishment of a Patient-derived Xenograft Model of Pancreatic Grade 3 Well-differentiated Neuroendocrine Tumor

Yosuke Kasai[1, 2], Grace E. Kim[2, 3], Byron Hann[2], David B. Donner[1, 2], Robert S. Warren[1, 2], Emily K. Bergsland[2, 4], Eric K. Nakakura[1, 2]
Department of [1]Surgery, [3]Pathology, and [4]Medicine, University of California, San Francisco, [2]UCSF Helen Diller Family Comprehensive Cancer Center

GO BACK TO KIOSK MENU

Fig. 1B. PDX tumors exhibited similar morphology, proliferation, and staining patterns of CgA, ATRX, p53, and Rb as the original liver metastasis.



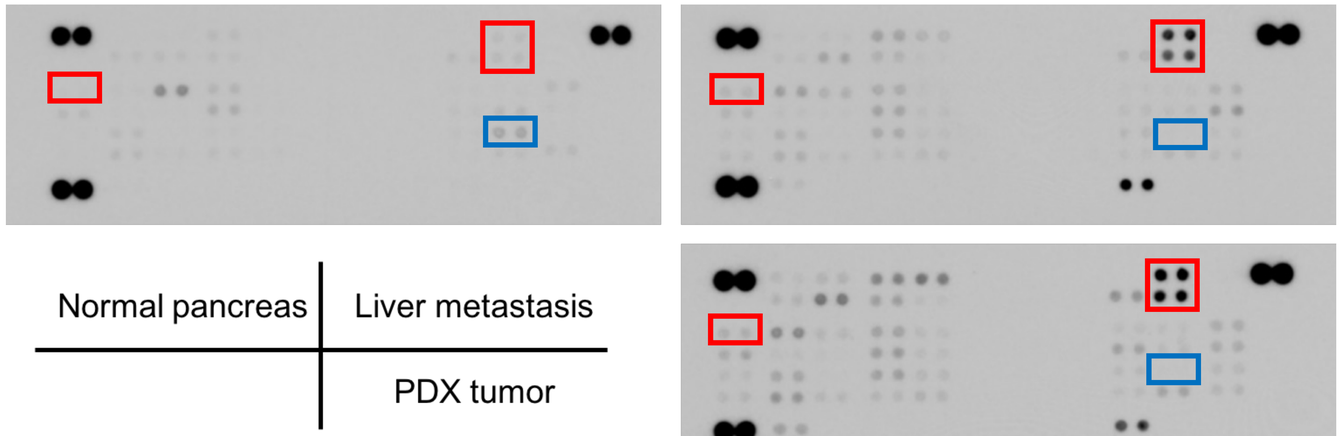
CLICK TO RETURN

Establishment of a Patient-derived Xenograft Model of Pancreatic Grade 3 Well-differentiated Neuroendocrine Tumor

Yosuke Kasai[1, 2], Grace E. Kim[2, 3], Byron Hann[2], David B. Donner[1, 2], Robert S. Warren[1, 2], Emily K. Bergsland[2, 4], Eric K. Nakakura[1, 2]
Department of [1]Surgery, [3]Pathology, and [4]Medicine, University of California, San Francisco, [2]UCSF Helen Diller Family Comprehensive Cancer Center

GO BACK TO KIOSK MENU

Fig. 1C, D. Phospho-kinase array showed a similar active kinase profile of the PDX tumor as the original liver metastasis, including enhanced phosphorylation of mTOR and p53 (S392 and S46) and attenuated phosphorylation of p27 (encoded by *CDKN1B* gene).



Ref	p38α	ERK1/2	JNK1/2/3	GSK-3α/β			p53 (S392)		Ref
	EGFR	MSK1/2	AMPKα1	Akt1/2/3		Akt1/2/3	p53 (S46)		
TOR	CREB	HSP27	AMPKα2	β-Catenin		p70S6K	p53 (S15)	c-Jun	
Src	Lyn	Lck	STAT2	STAT5a		p70S6K	RSK1/2/3	eNOS	
Fyn	Yes	Fgr	STAT6	STAT5b		STAT3	p27	PLC-γ1	
Hck	Chk-2	FAK	PDGFRβ	STAT5a/b		STAT3	WNK1	PYK2	
Ref	PRAS40			NC		HSP60			NC

Proteome Profiler™ Array Human Phospho-Kinase Array Kit (ARY003B)

from R&D SYSTEMS, Minneapolis, MN

👆 CLICK TO RETURN