



Ronald L. Heimark, PhD^{1, 2}; Brenna A. Rheinheimer, PhD^{1,2}; Taylor Riall, MD, PhD, FACS^{1,2}; Tun Jie, MD, MS, FACS^{1,2}

¹Department of Surgery, The University of Arizona, Tucson, AZ, USA

²The University of Arizona Cancer Center, Tucson, AZ, USA

Introduction

- Pancreatic cancers with acinar cell differentiation are rare (<2% of pancreatic cancers) and include acinar cell carcinomas and mixed acinar carcinomas with neuroendocrine or ductal differentiation. (1)
- Acinar carcinomas are distinct from other pancreatic neoplasms with unique clinical, morphological, gene mutations, and markers. Morphologically acinar carcinomas are characterized by cells with significant acinar differentiation.
- Somatic mutations described in acinar cell carcinomas include: *MEN1*, *SMAD4*, *JAK1*, *RB1*, *TP53*, *APC*, *ARID1A*, *GNAS*, *MLL3*, *PTEN*, *FAT4*, *CTNNB1*, *BRAF*, *ATM*, *BAP1*, *BRCA2*, *PALB2*, *RNF43*, *FAT2*, *TSC2*, and *MSH2*. (2,3)
- Acinar cell carcinomas with either ductal or neuroendocrine differentiation are thought to be a variation.
- Our understanding of the molecular pathology of mixed Acinar - Neuroendocrine Carcinomas is insufficient for clinical management and prediction of disease aggressiveness.
- Organoid culture models are and established approaches to identify and target pathways involved in pancreatic cancer. (4)

Case Report:

A female patient presented with a history of diarrhea. Imaging identified a lesion in the head of the pancreas, which upon biopsy was identified as a mixed Acinar-Neuroendocrine Carcinoma. This acinar cell carcinoma in the pancreatic head was surgically resected by a pancreaticoduodenectomy (4.7cm, Ki-67 labeling index 5%, pT3N1). Extensive perineural and vascular invasion involving the uncinated margin was found. A trypsin stain showed intracytoplasmic granules consistent with acinar differentiation. The tumor also showed expression of the neuroendocrine markers synaptophysin and chromogranin. The tumor was negative for gastrin, somatostatin, insulin and glucagon. A neuroendocrine tumor of the duodenum (0.7 cm) was also resected (Ki-67 labeling 3%, WHO 2, pT1N0) with strong synaptophysin positivity and patchy gastrin.

References

1. Wisnoski NC, Townsend CM, Nealon WH, Freeman JL, Riall T. 2008. 672 patients with acinar cell carcinoma of the pancreas: a population-based comparison to pancreatic adenocarcinoma. *Surgery* 144:141.
2. Jiao Y, Yonescu R, Offerhaus GJA, Kimstra DS, Maitra A, Eshleman JR et al., 2014 Whole-exome sequencing of pancreatic neoplasms with acinar differentiation. *J Pathol* 232:428.
3. Furukawa T, Sakamoto H, Takeuchi S, Kuboki Y, Yamamoto T, Harari M et al., 2015. Whole exome sequencing reveals recurrent mutations in *BRCA2* and *Fat* genes in acinar cell carcinomas of the pancreas. *Scientific Reports* 5:8829.
4. Boj SF, Hwang C, Baker LA, Chio C, Engle DD, Corbo, V. et al., 2015. Organoid models of human and mouse ductal pancreatic cancer. *Cell* 160: 324
5. Rondinelli B, Rosano D, Antonini E, Frenquelli M, Montanini L, Huang D, Segalla S, . Histone demethylase JARID1C inactivation triggers genomic instability in sporadic renal cancer. *J Clin Invest.* 125:4625.

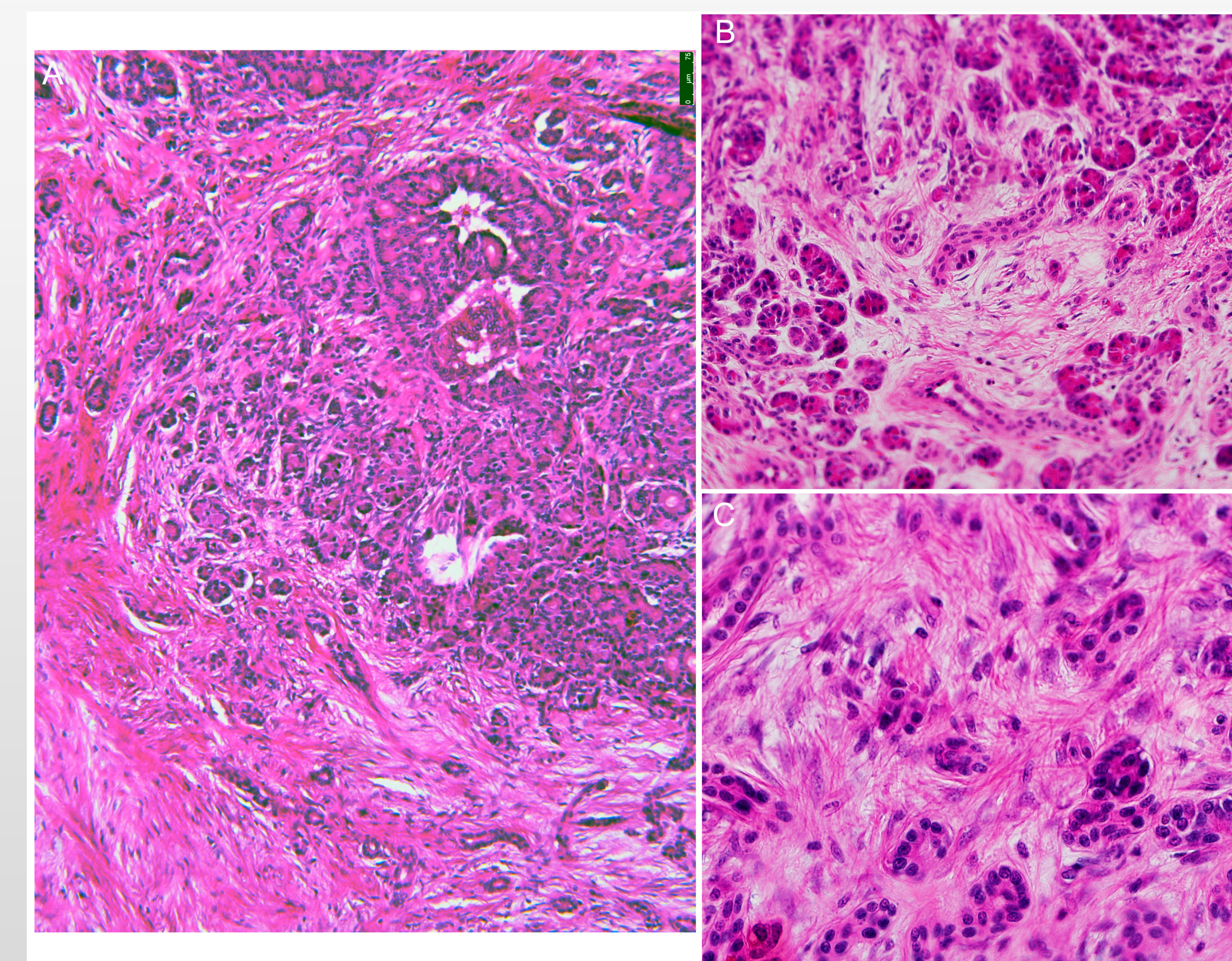
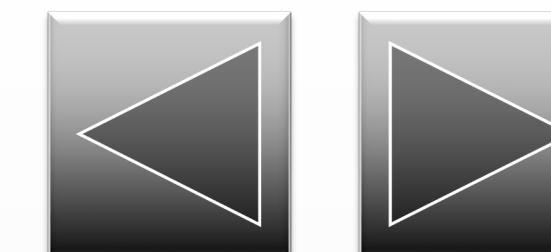


Figure 1. H&E stained section of a pancreatic mixed Acinar-Neuroendocrine tumor. Representative sections stained with haematoxylin and eosin showing both acinar carcinoma and neuroendocrine morphology. Note: extensive population of acinar structures surrounded by stromal cell population. (A) 10X; (B) 20X, (C) 40X.

CLICK TO GO BACK
TO KIOSK MENU

Pancreatic Mixed Acinar-Neuroendocrine Carcinoma: Genomic analysis and characterization of a patient-derived organoid culture



Ronald L. Heimark, PhD^{1,2}; Brenna A. Rheinheimer, PhD^{1,2}; Taylor Riall, MD, PhD, FACS^{1,2}; & Tun Jie, MD, MS, FACS^{1,2}

¹Department of Surgery, The University of Arizona, Tucson, AZ, USA

²The University of Arizona Cancer Center, Tucson, AZ, USA

Methods

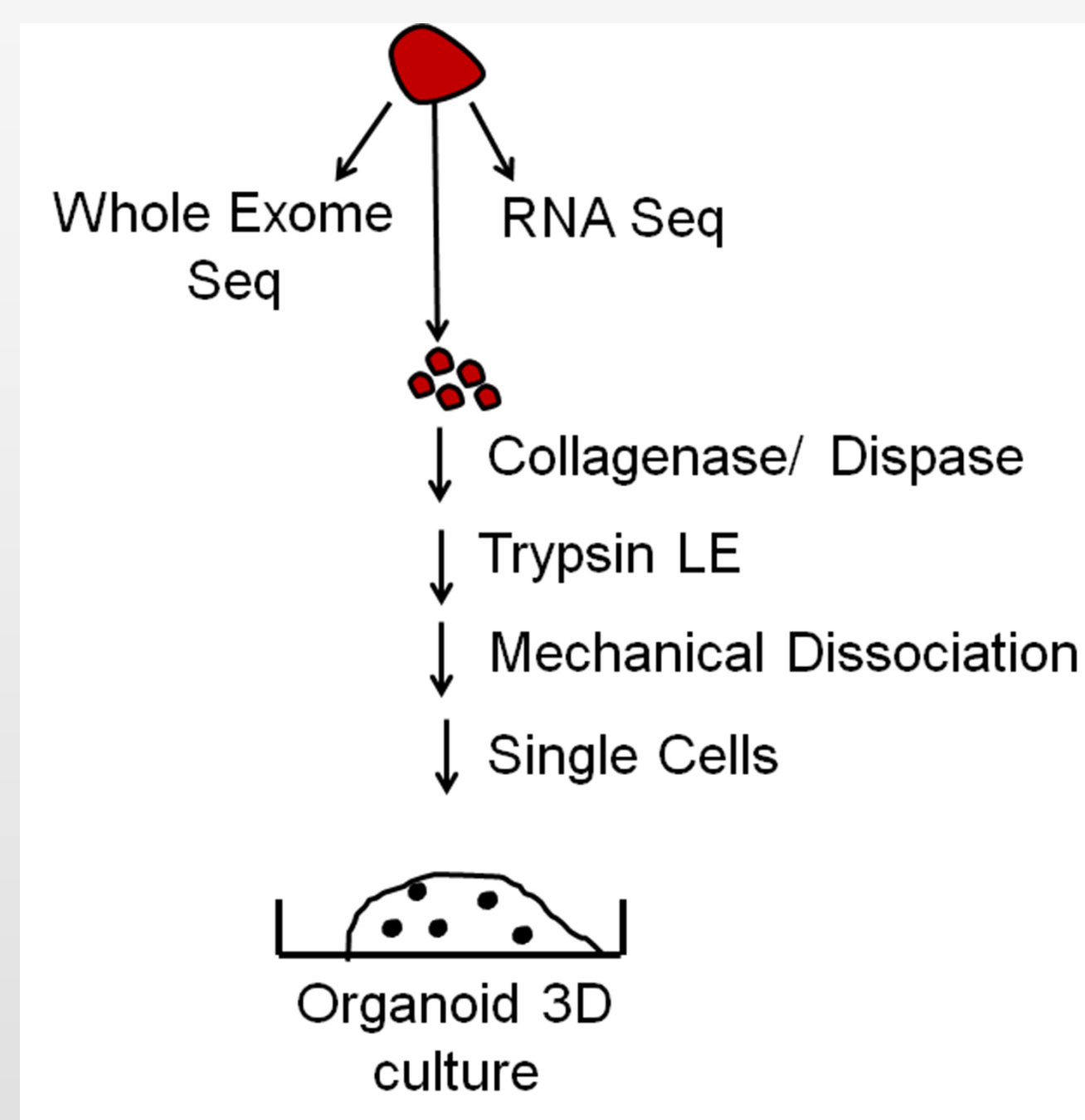


Figure 2. Approach to define the molecular pathology of pancreatic mixed Acinar-Neuroendocrine Carcinoma. De-identified fresh tumor samples were utilized to develop organoid cultures from a patient who underwent surgical resection without receiving preoperative therapy. Tissue was minced and digested with Collagenase/Dispase in DMEM/F12 medium at 37°C for a maximum of 16 hr. The material was further digested with TrypsinLE (GIBCO) for 15 min at 37°C, embedded in 50% GFR Matrigel, and cultured in human complete medium (4). Cultures were passaged by gently disrupting the Matrigel cultures and replating embedded in Matrigel (3D). Cultures showing robust growth were trypsinized with Trypsin LE and replated on 2% Matrigel coated dishes (2D). gDNA was isolated from snap frozen tumor tissue and the pANEC17 cells. Whole exome sequencing was performed using the Nextera Rapid Capture Exome Kit by Illumina on an Illumina HiSeq 2000/2500. The following criteria were used to define genetic variants: bidirectional, non-synonymous, 30X coverage, clean mapping in IGV, and a $\geq 10\%$ alternate allele frequency.

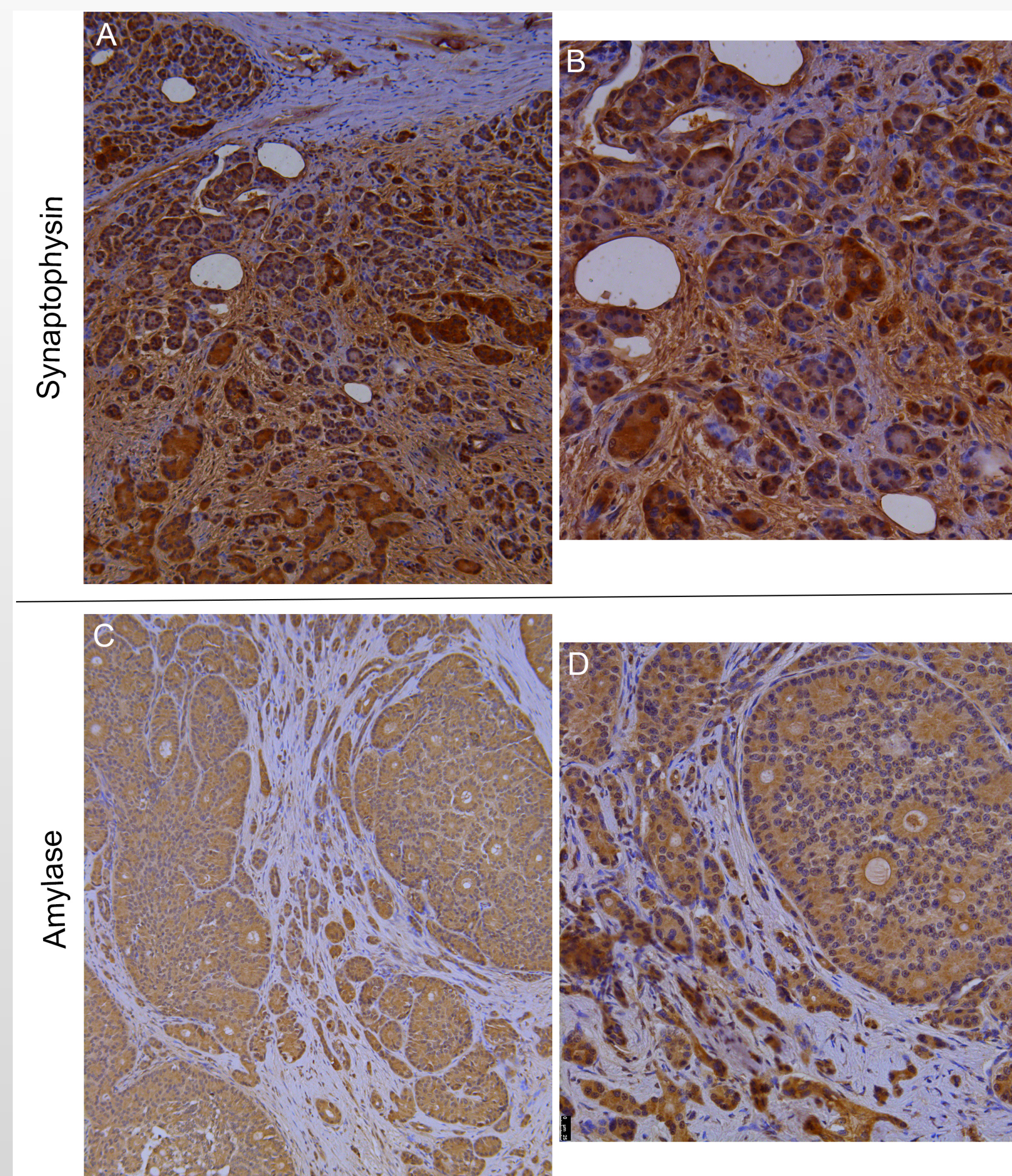


Figure 3. Synaptophysin and Amylase localization in patient tumor tissue. The immunoreactivity for the neuroendocrine marker synaptophysin (A) and the acinar marker amylase (B). **Note:** amylase is positive only in cells with acinar morphology. In contrast, synaptophysin is positive in both acinar cells and surrounding cells.

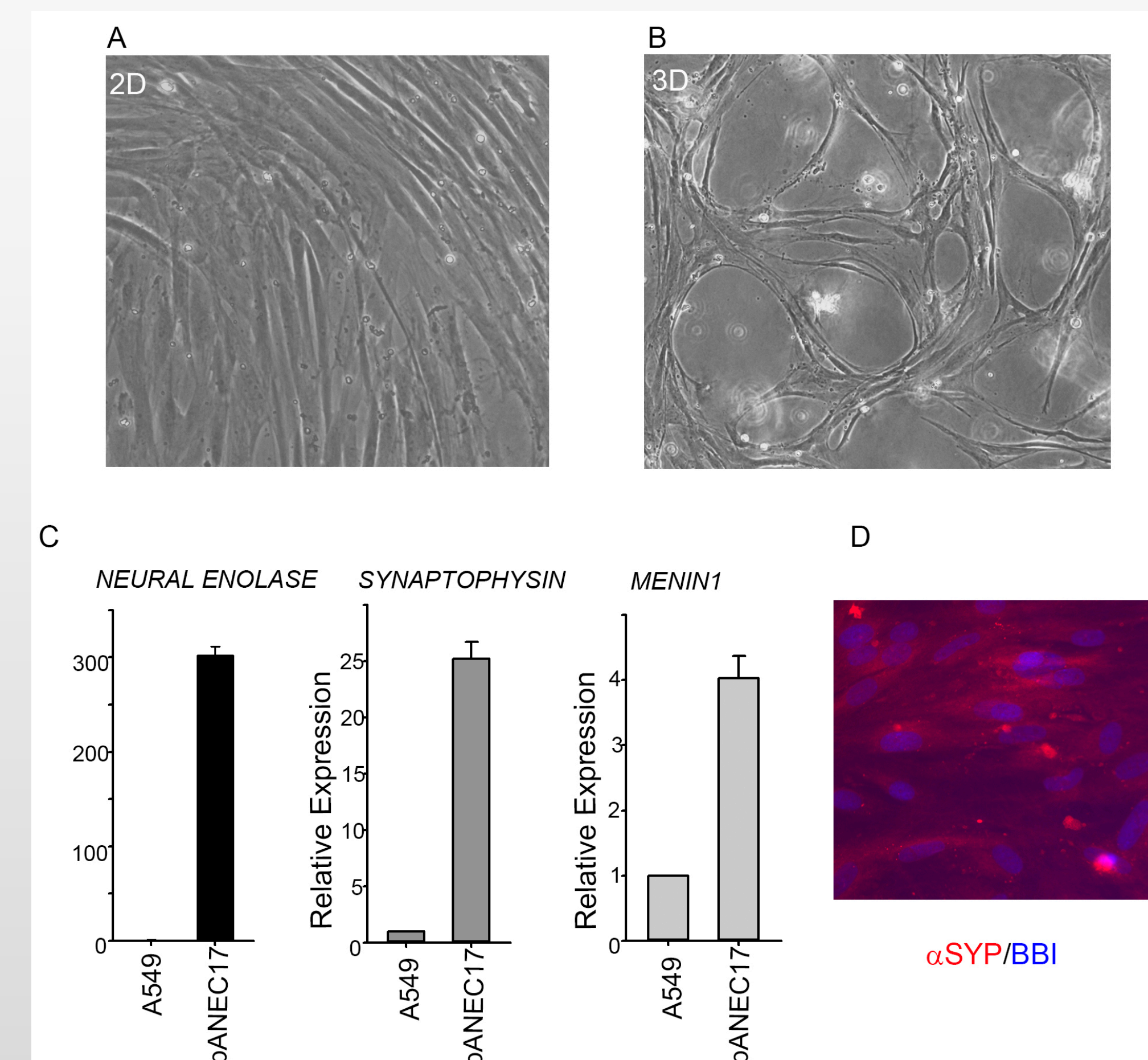
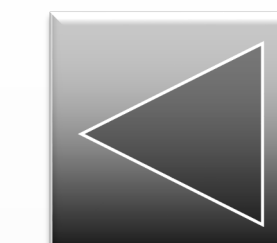


Figure 4. The pANEC17 cell line was propagated from the mixed Acinar-Neuroendocrine tumor specimen. Photomicrographs of the cell line is referred to as pANEC17 is shown in panel (A) 2D culture and panel (B) 3D culture. Gene expression (C) for *Neural Enolase*, *MENIN1* and *Synaptophysin* was analyzed by real time QRT/PCR and normalized to HPRT1. Relative expression is shown compared to the lung non-small cell line A549. (D) Immunofluorescence staining of synaptophysin and BBI in pANEC17 cells.

Pancreatic Mixed Acinar-Neuroendocrine Carcinoma: Genomic analysis and characterization of a patient-derived organoid culture



Ronald L. Heimark, PhD^{1,2}; Brenna A. Rheinheimer, PhD^{1,2}; Taylor Riall, MD, PhD, FACS^{1,2}; & Tun Jie, MD, MS, FACS^{1,2}

¹Department of Surgery, The University of Arizona, Tucson, AZ, USA

²The University of Arizona Cancer Center, Tucson, AZ, USA

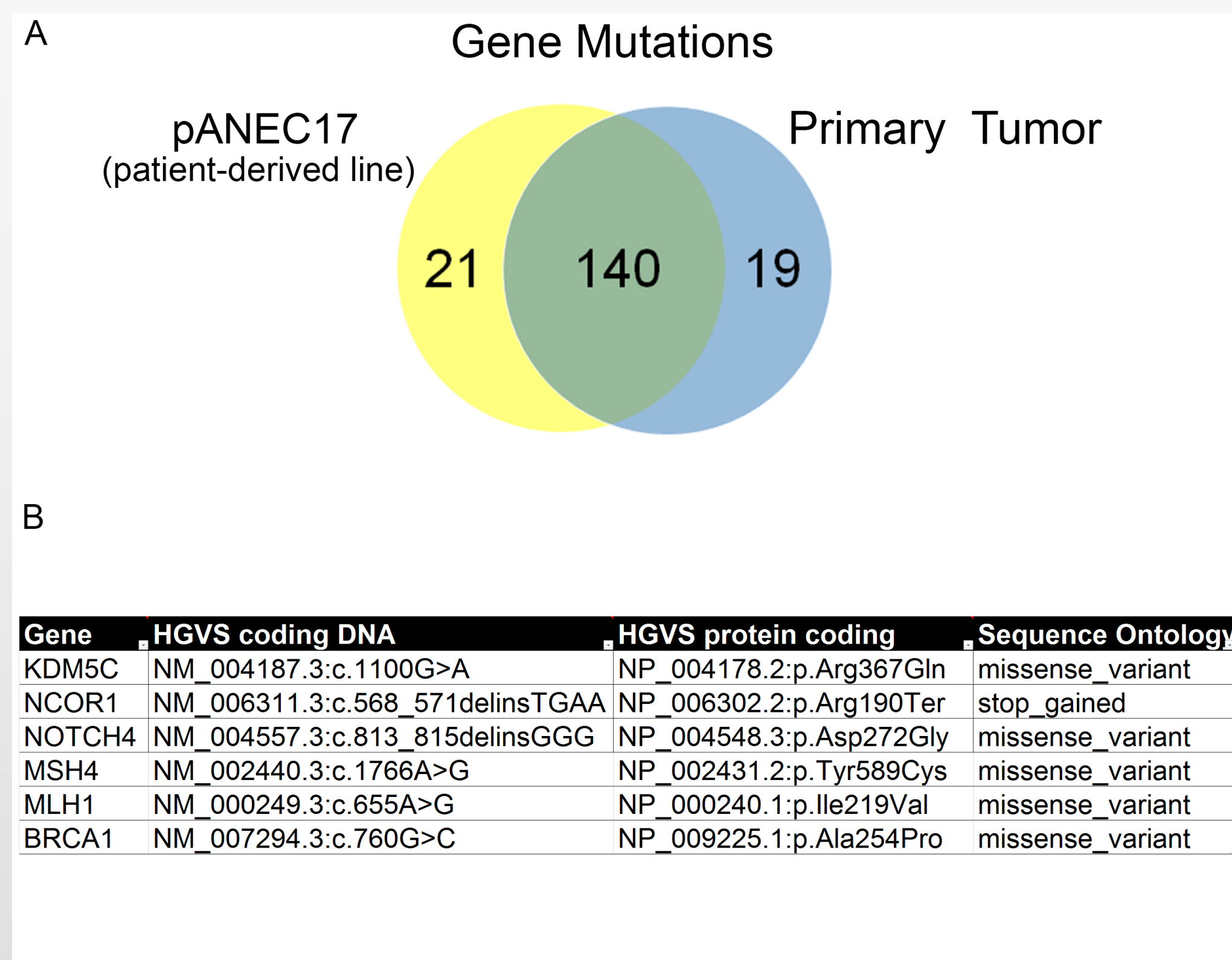


Figure 5. Overlap of mutated genes in primary tumor and tumor derived cell line (pANEC17). (A) Venn diagram showing the significant overlap of mutations found in the primary mixed Acinar Neuroendocrine Carcinoma and the tumor-derived neuroendocrine cell line (pANEC17) identified in whole exome sequencing. (B) Table of selected mutated genes present in both the primary tumor and tumor-derived cell line (pANEC17).

Conclusions

- The mutational landscape of this mixed Acinar Neuroendocrine Carcinoma highly overlaps with the tumor-derived cell line (pANEC17) and includes pathways of genomic instability.
- Neuroendocrine markers were found in acinar carcinoma cells and surrounding stromal cells.
- The histone demethylase KDM5c is mutated in the first PHD domain. Normally KDM5c represses neural genes. In cancer mutations in KDM5c can contribute to genomic instability.⁽⁵⁾

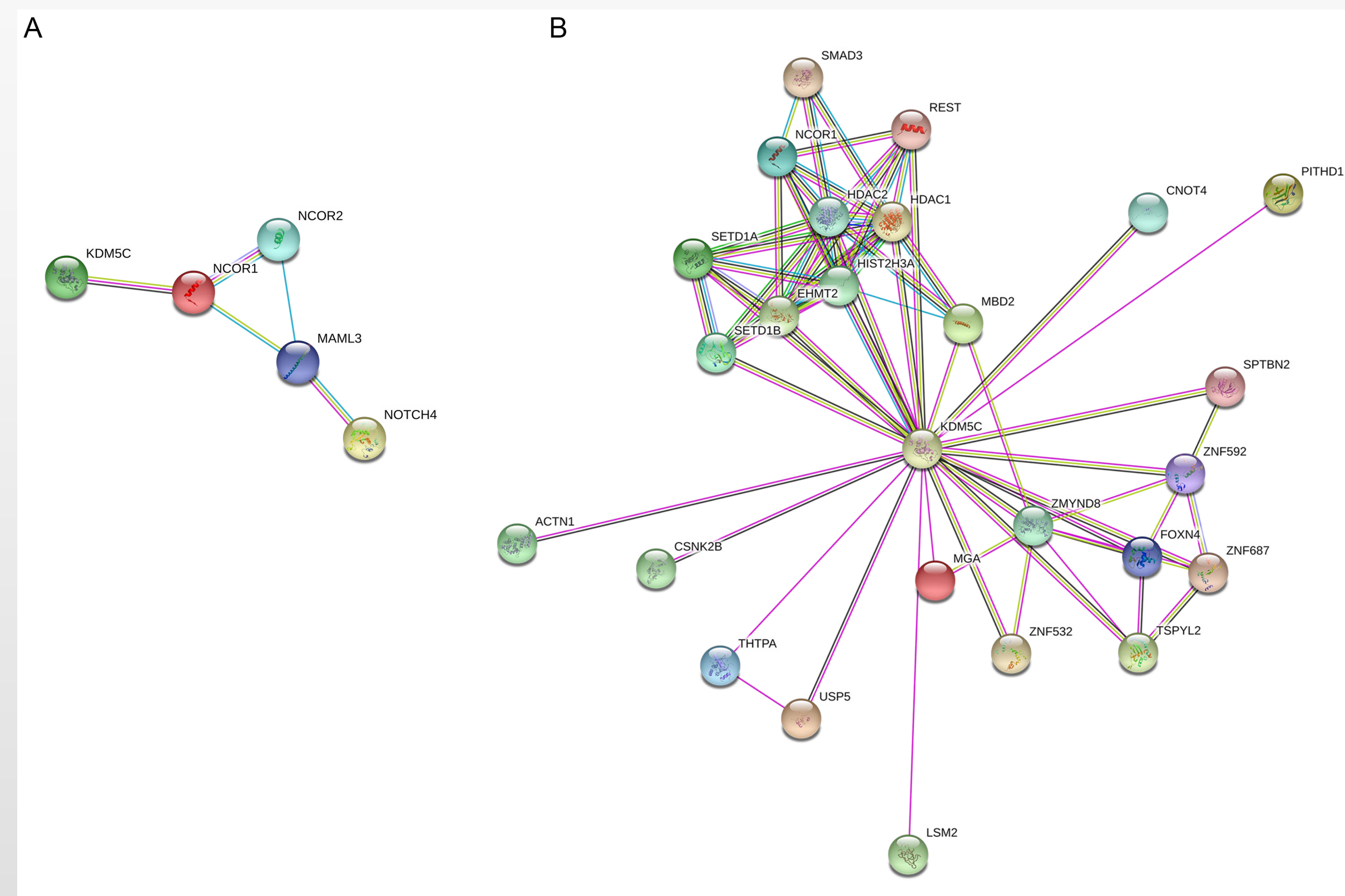


Figure 6. Network analysis of predicted interactions with KDM5c (Lysine-specific demethylase 5c). KDM5c is a histone demethylase that specifically demethylates K4 and K9 of histone 3 and has a central function in the histone code of enhancers and promoters. (A) Mutant KDM5c predicted interactions with other mutant genes identified in the whole exome sequencing data set. Interacting genes include NCOR1 and NCOR2 (nuclear receptor corepressor 1 and 2), MAML3 (mastermind like transcriptional coactivator 3) and Notch4. Mutations were identified in KDM5c, NOCR1 and Notch4. (B) The string interaction network for KDM5c predicted from evidence in the literature. KDM5c is involved in transcriptional repression of neural genes by recruiting HDACs and the REST complex to neuron-restrictive silencer elements in genes.

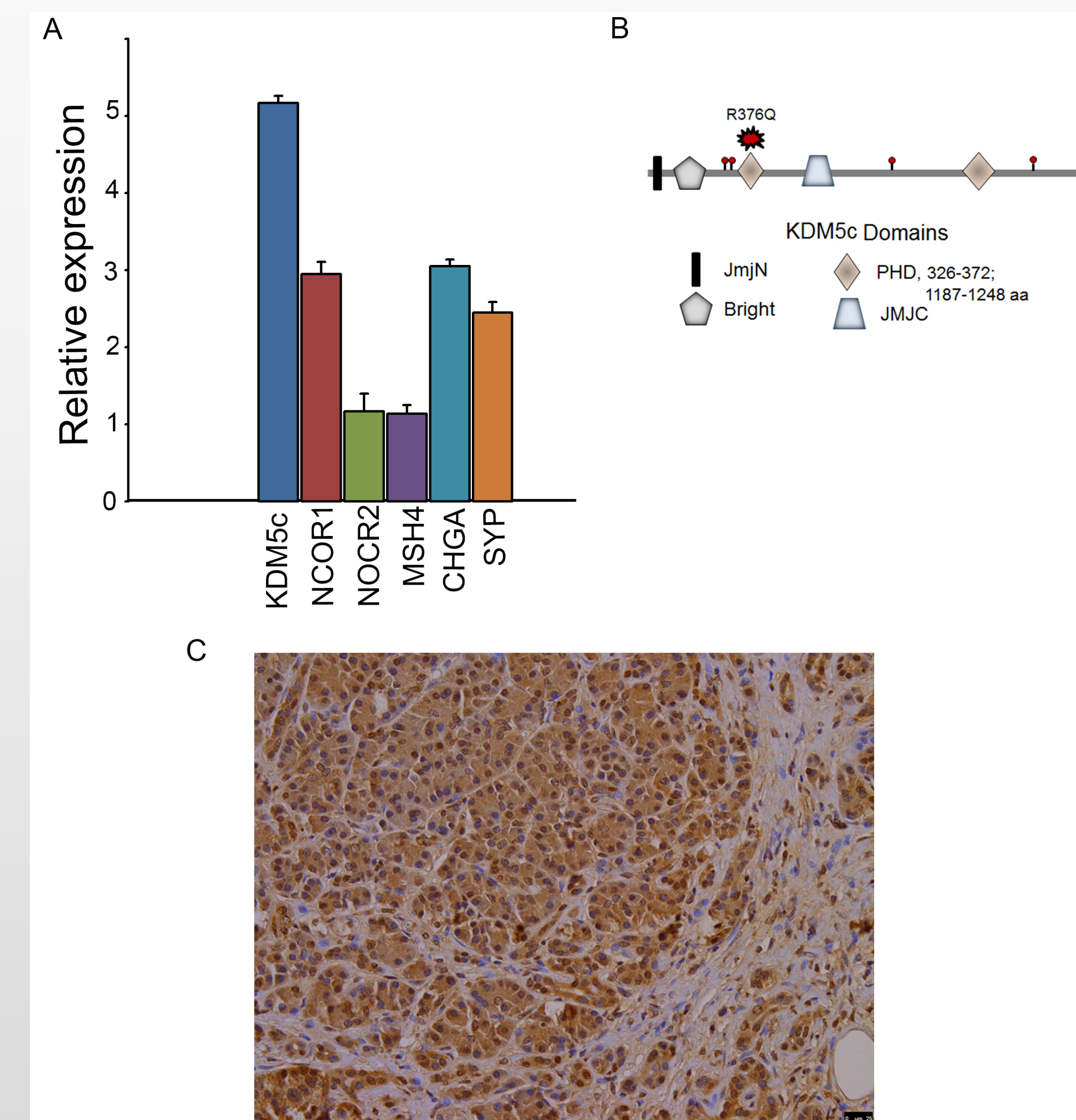


Figure 7. KDM5c expression in primary tumor and the derived cell line. (A) Quantitative RT/PCR was carried out and mRNA levels were normalized to A549 mRNA relative to *HPRT1*. (B) Model of protein domains in KDM5c. We observed a mutation at Arg 367 to Gln, which is in the 3' end of the first PHD domain. The PHD homeodomain is a zinc-finger-like motif found in nuclear proteins thought to be involved in chromatin-mediated transcriptional regulation. The PHD finger binds two zinc ions and is involved in protein-protein interaction. (C) Immunolabeling of KDM5c in the primary tumor shows mixed nuclear localization. KDM5c is located on the X-chromosome, but is not inactivated.