HHS Public Access

Author manuscript

*Gastroenterology*. Author manuscript; available in PMC 2018 June 03.

Published in final edited form as:

*Gastroenterology*. 2018 June ; 154(8): 2060–2063.e8. doi:10.1053/j.gastro.2018.02.026.

# Loss of Chromatin Remodeling Proteins and/or *CDKN2A* Associates With Metastasis of Pancreatic Neuroendocrine Tumors and Reduced Patient Survival Times

**Somak Roy, MD**[1], **William A. LaFramboise, PhD**[1], **Ta-Chiang Liu, MD PhD**[2], **Dengfeng Cao, MD PhD**[2], **Alyssa Luvison, BS**[1], **Caitlyn Miller, BS**[1], **Maureen A. Lyons, MS**[1], **Roderick J. O'Sullivan, PhD**[3], **Amer H. Zureikat, MD**[4], **Melissa E. Hogg, MD**[4], **Allan Tsung, MD**[4], **Kenneth K. Lee, MD**[4], **Nathan Bahary, MD PhD**[5], **Randall E. Brand, MD**[5], **Jennifer S. Chennat, MD**[5], **Kenneth E. Fasanella, MD**[5], **Kevin McGrath, MD**[5], **Marina N Nikiforova, MD**[1], **Georgios I. Papachristou, MD, PhD**[5], **Adam Slivka, MD, PhD**[5], **Herbert J. Zeh, MD**[4,*], and **Aatur D. Singhi, MD PhD**[1,*]

[1]Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA

[2]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO

[3]Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA

[4]Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA

[5]Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA

## Abstract

Despite prognostic grading and staging systems, it is a challenge to predict outcomes for patients with pancreatic neuroendocrine tumors (PanNETs). Sequencing studies of PanNETs have identified alterations in death domain-associated protein (DAXX) and ATRX chromatin remodeler (ATRX). In tumors, mutations in *DAXX* or *ATRX* and corresponding loss of protein expression correlate with shorter times of disease-free survival and disease-specific survival of patients. However, DAXX or ATRX proteins were lost in only 50% of distant metastases analyzed. We performed whole-exome sequencing analyses of 20 distant metastases from 20 patients with a single non-syndrome, non-functional PanNET. We found distant metastases contained alterations in *MEN1* (n=8), *ATRX* (n=5), *DAXX* (n=5), *TSC2* (n=3), and *DEPDC5* (n=3). We found copy

[*]**Correspondence to:** Herbert J. Zeh, MD, UPMC Hillman Cancer Center, 5115 Centre Avenue, Pittsburgh, PA 15232, zehxhx@upmc.edu, Aatur D. Singhi, MD PhD, UPMC Presbyterian Hospital, 200 Lothrop Street, Room A616.2, Pittsburgh, PA 15213, Phone: 412-864-1508, singhiad@upmc.edu.



**Disclosure/conflict of interest:** The authors have no conflicts of interest to declare.

**Author Contributions**

Study Concept and Design: SR, WAF, HZ and ADS
Acquisition of Data: SR, WAF, TCL, DC, AL, CM, MAL, RJO, AHZ, MEH, AT, KKL, NB, REB, KM, MNN, AS, HJZ, and ADS
Analysis and Interpretation of Data: SR, WAF, HZ and ADS
Drafting of the Manuscript: SR, WAF, HZ and ADS

number loss of *CDKN2A* in 15 metastases (75%) and alterations in genes that regulate chromatin remodeling including *SETD2* (n=4), *ARID1A* (n=2), *CHD8* (n=2), and *DNMT1* (n=2). In a separate analysis of 347 primary PanNETs, we found loss or deletion of *DAXX* and *ATRX*, disruption of SETD2 function (based on loss of H3K36me3), loss of ARID1A expression or deletions in *CDKN2A* in 81% of primary PanNETs with distant metastases. Among patients with loss or deletion of at least 1 of these proteins or genes, 39% survived disease free for 5 years and 44% had disease-specific survival times of 10 years. Among patients without any of these alterations, 98% survived disease free for 5 years and 95% had disease-specific survival times of 10 years. Therefore, primary PanNETs with loss of DAXX, ATRX, H3K36me3, ARID1A, and/or *CDKN2A* associate with shorter survival times of patients. Our findings indicate that alterations in chromatin remodeling genes and *CDKN2A* contribute to metastasis of PanNETs.

**Keywords**

pancreas; prognosis; prognostic factor; risk

Pancreatic neuroendocrine tumors (PanNETs) are a heterogeneous group of neoplasms with increasing incidence and ill-defined pathobiology. While most PanNETs are indolent and remain stable for years, a subset may behave aggressively and metastasize widely. Thus, the frequent detection of PanNETs presents a treatment dilemma. Current prognostic parameters and systems, such as tumor size and World Health Organization (WHO) grade, are susceptible to interpretation errors, sampling issues and, in a subset of PanNETs, do not accurately reflect the clinical behavior of these neoplasms.[1–4] Hence, additional markers are needed to improve the prognostic classification of PanNETs.

Recently, whole-exome and whole-genome sequencing studies have focused on identifying recurrent genetic alterations in primary PanNETs.[5, 6] Among these alterations, the most commonly mutated genes are *MEN1, DAXX* and *ATRX*. Death domain-associated protein (*DAXX*) and alpha-thalassemia/mental retardation X-linked (*ATRX*) genes encode for proteins that participate in chromatin remodeling at telomeres. Mutations in these genes are associated with loss of nuclear expression of their respective proteins by immunohistochemistry and correlate with alternative lengthening of telomeres (ALT), a telomerase-independent telomere maintenance mechanism.[7] In addition, loss of DAXX and/or ATRX is associated with shorter times of disease-free survival (DFS) and disease-specific survival (DSS).[8–10] Consequently, DAXX and/or ATRX loss is considered to be a driver of metastasis. However, only 50% of distant metastases demonstrate loss of DAXX and/or ATRX.

In contrast to primary PanNETs, the genetic landscape of metastatic PanNETs remains relatively unknown and, therefore, we hypothesized that additional genetic alterations other than those involving *DAXX* and *ATRX* account for the metastatic progression of PanNETs and may serve as useful prognostic markers. Thus, we performed whole-exome sequencing of 20 distant metastases from 20 patients with a solitary, non-syndrome and non-functional PanNET (Supplementary Materials and Methods). Similar to sequencing studies of primary PanNETs, whole-exome sequencing of metastatic PanNETs revealed frequent genomic

alterations in *MEN1* (n=8), *ATRX* (n=5), *DAXX* (n=5), *TSC2* (n=3), and *DEPDC5* (n=3) (Supplementary Table 1 and Supplementary Figure 1).[5, 6] Inactivating mutations in *DAXX* and *ATRX* were mutually exclusive and correlated with loss of corresponding protein expression and the presence of ALT by telomere FISH. In addition, as described by Heaphy et al[7], 2 DAXX-negative and 2 ATRX-negative metastatic PanNETs lacked mutations in *DAXX* and *ATRX*, respectively. In contrast to primary PanNETs, *MEN1* was not the most commonly altered gene in metastatic PanNETs. *CDKN2A* copy number loss was found in 15 (75%) cases. Furthermore, genomic alterations in chromatin remodeling genes, such as *SETD2* (n=4), *ARID1A* (n=2), *CHD8* (n=2) and *DNMT1* (n=2), were seen in 10 (50%) metastatic PanNETs.

Considering genomic alterations in *SETD2, ARID1A* and *CDKN2A* were previously described in primary PanNETs, but at a significantly lower prevalence than in metastatic PanNETs, the status of *SETD2, ARID1A* and *CDKN2A* was reevaluated using orthogonal methods. The *SETD2* gene encodes for a histone methyltransferase that is specific for H3 lysine 36 trimethylation (H3K36me3) and loss-of-function mutations result in the absence of H3K36me3 expression by immunohistochemistry.[11] *ARID1A* inactivating mutations are associated with loss of the corresponding protein.[12] Genomic deletions in *CDKN2A* can be assayed using dual-color FISH.[13] An analysis of the sequenced metastatic PanNETs revealed loss of H3K36me3 and ARID1A by immunohistochemistry and deletions for *CDKN2A* by dual-color FISH had a 100% concordance with alterations in their respective genes (Supplementary Figure 2).

In order to determine the prognostic significance of *SETD2, ARID1A* and *CDKN2A* alterations in relationship to DAXX/ATRX loss, the status of DAXX, ATRX, H3K36me3, ARID1A and *CDKN2A* was evaluated in 347 solitary, non-syndromic, primary PanNETs (Supplementary Tables 2 and 3). Loss or deletion of DAXX/ATRX, H3K36me3, ARID1A, *CDKN2A* was identified in 80 (23%), 28 (8%), 10 (3%) and 25 (7%) of primary PanNETs, respectively, and associated with larger mean tumor size, higher WHO grade, lymphovascular invasion, higher pathologic tumor stage, synchronous distant metastases and metachronous distant metastases ($p < 0.05$). Of note, DAXX/ATRX loss correlated with deletion in *CDKN2A*, but not the absence of H3K36me3 or ARID1A.

Overall, DAXX, ATRX, H3K36me3, ARID1A and/or *CDKN2A* loss or deletion was identified in 112 (32%) PanNETs. Further, loss or deletion of at least 1 marker was detected in 81 (81%) primary PanNETs from patients, who either had synchronous metastases (41 of 55, 75%) or developed metachronous metastases (without synchronous metastases) on follow-up (40 of 45, 89%). The DFS and DSS for patients with PanNETs demonstrating either loss or deletion in DAXX, ATRX, H3K36me3, ARID1A and/or *CDKN2A* were 63% at 3-years and 39% at 5-years ($p < 0.001$, $X^2 = 142.5$), and 75% at 5-years and 44% at 10-years ($p < 0.001$, $X^2 = 53.9$), respectively (Figure 1 and Supplementary Figure 3). Conversely, patients with PanNETs that lacked alterations in DAXX, ATRX, H3K36me3, ARID1A and *CDKN2A* had a DFS of 99% at 3-years and 98% at 5-years, and DSS of 96% at 5-years and 95% at 10-years. Univariate and multivariate Cox regression analysis were used to determine the prognostic significance of these 5 markers. Loss or deletion in any 1

marker was an independent prognostic factor for DFS with a hazard ratio (HR) of 14.44 ($p < 0.001$) and DSS with a HR of 3.12 ($p = 0.006$) (Table 1).

Notwithstanding, our study has a number of limitations. It is retrospective by design and not all patients received the same form of treatment. Among patients with primary PanNETs, 8% underwent enucleation or central pancreatectomy, and, as a result, regional lymphadenectomy may be suboptimal. Removing these patients from our analyses would have little impact on the statistical correlations associated with DAXX, ATRX, H3K36me3, ARID1A and *CDKN2A* status. Of note, enucleation and central pancreatectomy are typically done in the setting of small PanNETs (≤2 cm) because they often have an indolent clinical course.[14] However, studies suggest a subset of small PanNETs can behave aggressively.[2, 3] Within our cohort, 2% of small PanNETs developed distant metastases and in each case harbored loss or deletion of at least 1 marker. Although additional studies are necessary, loss or deletion in DAXX, ATRX, H3K36me3, ARID1A and/or *CDKN2A* in preoperative biopsies may indicate an increased risk of developing metastatic disease and, in turn, prompt a change in surgical management to ensure complete regional lymph node dissection.

In summary, metastatic PanNETs not only harbor frequent genetic alterations in *MEN1, DAXX* and *ATRX*, but also in *SETD2, ARID1A* and *CDKN2A*. Loss or deletion of DAXX/ATRX, H3K36me3 (as a surrogate for *SETD2*), ARID1A and *CDKN2A* in primary PanNETs correlated with several adverse clinicopathologic features. In addition, loss or deletion of at least 1 marker was associated with shorter DFS and DSS times, and a negative, prognostic factor for DFS and DSS, independent of tumor size and WHO grade. While further studies are required, the assessment of these 5 markers by immunohistochemistry and FISH offers an objective method of evaluating prognostic risk using pathologic specimens. Moreover, our observations highlight the potential role of chromatin remodeling genes and *CDKN2A* in the metastatic progression of PanNETs.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

**Funding support:** This project was supported in part by a grant from the National Pancreas Foundation, Western Pennsylvania Chapter (to A. D. Singhi).

## References

1. Reid MD, et al. Mod Pathol. 2016; 29:93.
2. Kuo EJ, et al. Ann Surg Oncol. 2013; 20:2815–21. [PubMed: 23771245]
3. Haynes AB, et al. Arch Surg. 2011; 146:534–8. [PubMed: 21576607]
4. Diaz Del Arco C, et al. Diagn Cytopathol. 2017; 45:29–35. [PubMed: 27863178]
5. Jiao Y, et al. Science. 2011; 331:1199–203. [PubMed: 21252315]
6. Scarpa A, et al. Nature. 2017; 543:65–71. [PubMed: 28199314]
7. Heaphy CM, et al. Science. 2011; 333:425. [PubMed: 21719641]
8. Singhi AD, et al. Clin Cancer Res. 2017; 23:600–609. [PubMed: 27407094]

9. Kim JY, et al. Clin Cancer Res. 2017; 23:1598–1606. [PubMed: 27663587]
10. Marinoni I, et al. Gastroenterology. 2014; 146:453–60. e5. [PubMed: 24148618]
11. Ho TH, et al. Oncogene. 2016; 35:1565–74. [PubMed: 26073078]
12. Gibson WJ, et al. Nat Genet. 2016; 48:848–55. [PubMed: 27348297]
13. Singhi AD, et al. Mod Pathol. 2016; 29:14–24. [PubMed: 26493618]
14. Kulke MH, et al. J Natl Compr Canc Netw. 2015; 13:78–108. [PubMed: 25583772]

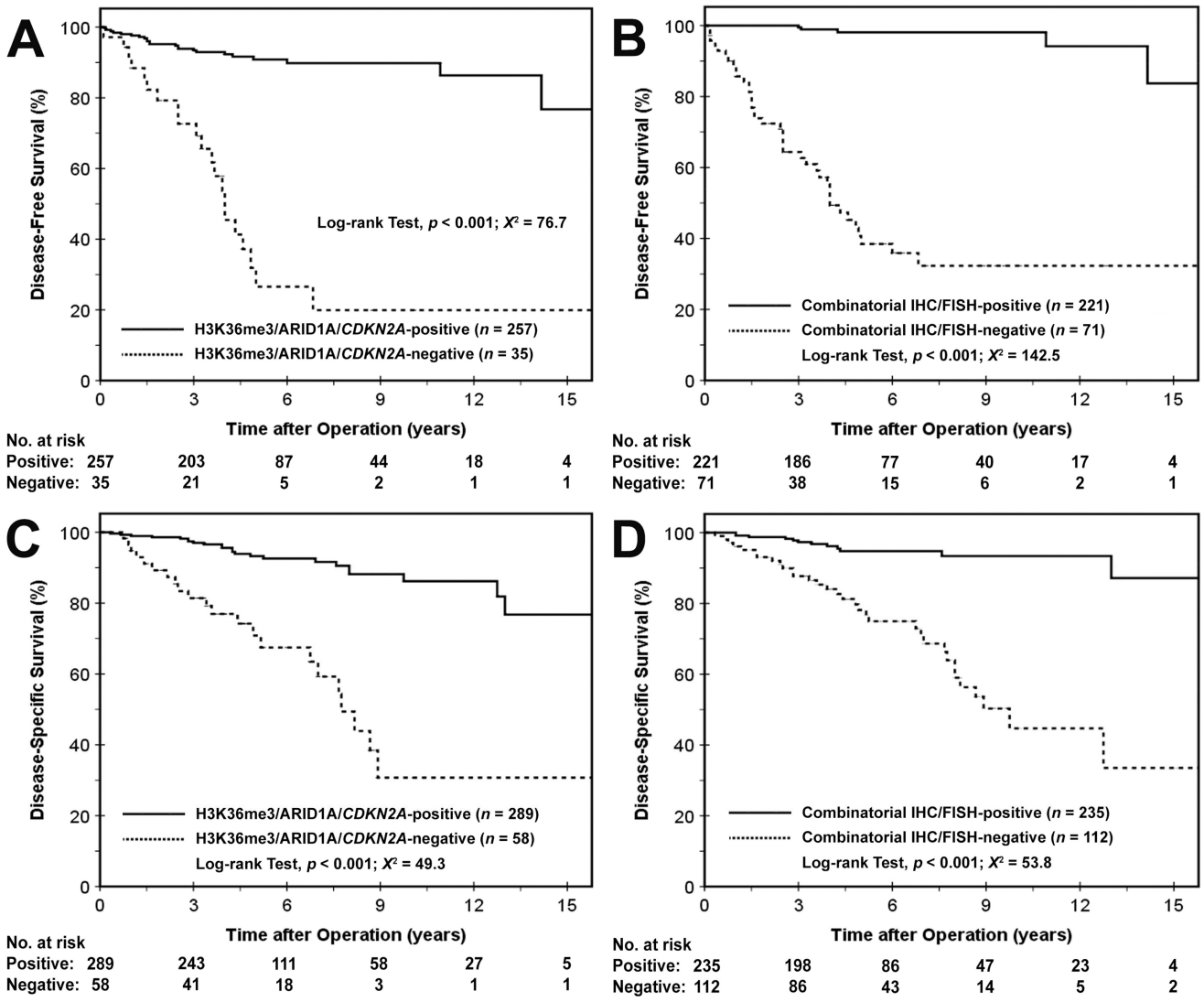


**Figure 1.**

Kaplan-Meier curves comparing the cumulative probability of disease-free survival (A and B, n=282) and disease-specific survival (C and D, n=347) after surgical resection among PanNET patients with regards to H3K36me3/ARID1A/*CDKN2A* and DAXX/ATRX/ H3K36me3/ARID1A/*CDKN2A* status. Disease-free survival was calculated in patients that did not present with distant metastases at the time of surgical resection.

**Table 1**

Univariate and multivariate Cox regression analysis for disease-free survival and disease-specific survival.

| Patient or Tumor Characteristics | Univariate Cox Regression Analysis | | | | Multivariate Cox Regression Analysis | | | |
|---|---|---|---|---|---|---|---|---|
| | Disease-Free Survival HR (95% CI) | *p* | Disease-Specific Survival HR (95% CI) | *p* | Disease-Free Survival HR (95% CI) | *p* | Disease-Specific Survival HR (95% CI) | *p* |
| Gender, male vs. female | 1.10 (0.61 – 1.99) | 0.752 | 0.94 (0.52 – 1.68) | 0.824 | | | | |
| Age, years | **1.03 (1.01 – 1.06)** | **0.008** | **1.03 (1.00 – 1.05)** | **0.038** | | | | |
| Tumor size, > 2.0 cm vs. ≤ 2.0 cm | **37.79 (5.20 – 274.50)** | **< 0.001** | **27.57 (3.80 – 200.16)** | **0.001** | CRIP**10.30 (1.39 – 76.64)** | **0.023** | 5.21 (0.69 – 39.62) | 0.111 |
| Functional vs. nonfunctional | 0.18 (0.02 – 1.28) | 0.086 | 0.58 (0.18 – 1.89) | 0.584 | | | | |
| Location, head and uncinate vs. body and tail | 1.25 (0.69 – 2.27) | 0.459 | 1.67 (0.93 – 2.99) | 0.088 | | | | |
| WHO grade, G2 or G3 vs. G1 | **4.74 (2.50 – 8.98)** | **< 0.001** | **5.79 (2.91 – 11.53)** | **< 0.001** | 1.54 (0.77 – 3.07) | 0.215 | 2.05 (0.99 – 4.27) | 0.054 |
| Lymphovascular invasion, presence vs. absence | **6.60 (3.38 – 12.90)** | **< 0.001** | **11.73 (4.91 – 28.00)** | **< 0.001** | | | | |
| Perineural invasion, presence vs. absence | **5.43 (2.95 – 9.98)** | **< 0.001** | **4.05 (2.23 – 7.35)** | **< 0.001** | | | | |
| Tumor stage (pT), pT3 vs. pT1 and pT2 | **5.51 (3.01 – 10.08)** | **< 0.001** | **8.60 (4.23 – 17.47)** | **< 0.001** | | | | |
| Lymph node metastasis, presence vs. absence | **5.31 (2.84 – 9.93)** | **< 0.001** | **5.21 (2.69 – 10.10)** | **< 0.001** | 1.56 (0.80 – 3.04) | 0.190 | **2.12 (1.07 – 4.20)** | **0.031** |
| Distant metastases at presentation, presence vs. absence | | | **10.02 (5.43 – 18.49)** | **< 0.001** | | | **3.64 (1.91 – 6.91)** | **< 0.001** |
| DAXX/ATRX/H3K36me3/ARID1A/*CDKN2A*, loss/deletion vs. preserved/wild type | **35.45 (13.91 – 90.34)** | **< 0.001** | **9.55 (4.58 – 19.91)** | **< 0.001** | **14.43 (5.37 – 38.75)** | **< 0.001** | **3.12 (1.39 – 7.00)** | **0.030** |