Published in final edited form as:

Hum Pathol. 2012 April ; 43(4): 585–591. doi:10.1016/j.humpath.2011.06.009.

Loss of expression of the SWI/SNF chromatin remodeling subunit BRG1/SMARCA4 is frequently observed in intraductal papillary mucinous neoplasms (IPMNs) of the pancreas

Marco Dal Molin, MD¹, Seung-Mo Hong, MD¹, Sachidanand Hebbar, PhD¹, Rajni Sharma, MSc PhD¹, Francesca Scrimieri, PhD¹, Roeland de Wilde, MD¹, Skye C Mayo, MD MPH², Michael Goggins, MD^{1,3,4}, Christopher L Wolfgang, MD PhD², Richard D Schulick, MD^{2,3}, Ming-Tseh Lin, MD¹, James R. Eshleman, MD PhD^{1,3}, Ralph H Hruban, MD^{1,3}, Anirban Maitra, MBBS^{1,3}, and Hanno Matthaei, MD^{1,5}

¹The Sol Goldman Pancreatic Cancer Research Center, Department of Pathology, The Johns Hopkins University, Baltimore, USA

²The Sol Goldman Pancreatic Cancer Research Center, Department of Surgery, The Johns Hopkins University, Baltimore, USA

³The Sol Goldman Pancreatic Cancer Research Center, Department of Oncology, The Johns Hopkins University, Baltimore, USA

⁴The Sol Goldman Pancreatic Cancer Research Center, Department of Medicine, The Johns Hopkins University, Baltimore, USA

⁵The Sol Goldman Pancreatic Cancer Research Center, Department of General, Visceral, Thoracic, and Vascular Surgery, University of Bonn, Germany

Abstract

A better molecular characterization of Intraductal Papillary Mucinous Neoplasm (IPMN), the most frequent cystic precursor lesion of pancreatic adenocarcinoma, may have a pivotal role in its early detection and in the development of effective therapeutic strategies. *BRG1*, a central component of the chromatin remodeling complex SWI/SNF regulating transcription, is inactive in several malignancies. In this study we evaluate the Brg1 expression in IPMN in order to better understand its role in the pancreatic carcinogenesis. Tissue microarrays (TMAs) of 66 surgically resected IPMNs were immunolabeled for the Brg1 protein. Expression patterns were then correlated with clinicopathologic parameters. Normal pancreatic epithelium strongly immunolabeled for Brg1. Reduced Brg1 expression was observed in 32 (53.3%) of the 60 evaluable IPMN lesions and occurred more frequently in high-grade IPMNs (13 of 17 showed loss; 76%) compared to intermediate-grade (15 of 29 showed loss; 52%) and low-grade IPMNs (4 of 14 showed loss; 28%) ($p=0.03$). A complete loss of Brg1 expression was observed in 5 of the 60 (8.3%) lesions. Finally, a decrease in Brg1 protein expression was furthermore found in a low-passage non-invasive IPMN cell line by Western blot analysis. We did not observe correlation between Brg1

© 2011 Elsevier Inc. All rights reserved.

Corresponding author: Anirban Maitra, MBBS, Professor of Pathology and Oncology, Affiliate, McKusick-Nathans Institute of Genetic Medicine, The Sol Goldman Pancreatic Cancer Research Center, CRB-2, Suite 345, Johns Hopkins University School of Medicine, 1550 Orleans Street, Baltimore MD 21231, Phone: (410) 502 8191 (Direct)/(410) 955 3511; Fax: (410) 614 0671.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

expression and IPMN subtype or with location of the cyst. We provide first evidence that Brg1 expression is lost in noninvasive cystic precursor lesions of pancreatic adenocarcinoma.

Keywords

BRG1; IPMN; pancreatic cancer

Introduction

The expanding usage of abdominal imaging technologies is uncovering an increasing number of asymptomatic pancreatic cystic lesions, including cystic precursors of pancreatic cancer. In this scenario, Intraductal Papillary Mucinous Neoplasms (IPMNs) represent the most frequent cystic precursor lesions of pancreatic cancer and they have been associated with malignancy in about one third of patients¹⁻⁴. Although the malignant potential of IPMNs is unequivocal, the natural history of this disease is still incompletely understood.

Numerous molecular alterations that are thought to contribute to the development and progression of IPMNs have been described⁵. Those include activating *KRAS2* mutations⁶ as well as inactivation of *CDKN2A/p16* and *TP53*^{7;8}. Interestingly *SMAD4/DPC4* inactivation is not usually observed in non-invasive IPMN, as opposed to pancreatic adenocarcinoma (PDAC)⁹. Somatic mutations of the *STK11/LKB1* gene, which is responsible for Peutz-Jeghers Syndrome, are also reported in IPMN, as well as mutations of *PIK3CA*, which represents a finding rarely found in classic PDAC^{10;11}. Furthermore, IPMN gene expression profiling studies show differential up-or down regulation of numerous genes, compared to PDAC¹². Chromosomal imbalances are also a common feature of IPMN and have been shown by global profiling approaches to accumulate gradually, in parallel with the increasing grades of dysplasia. Particular chromosomal losses (5q, 6q, 11q) are more commonly observed in high-grade IPMN compared to PDAC, whereas others (18p, 15q, 18q, 22q) occur in both entities at a similar rate¹³⁻¹⁶.

A facilitative chromatin state, which allows for ready access to transcription factors, is essential for gene expression. Consequently, It has been shown that deregulation of the chromatin structure may have a severe impact on cellular processes and may contribute to cancer development¹⁷.

Brg1 is an ATPase/helicase and constitutes the catalytic subunit of the SWI/SNF chromatin remodeling complex. This complex has been shown to disrupt the adhesion of histone components and DNA, thereby giving transcription factors access to their target genes. Several studies have demonstrated the tumor suppressive nature of *BRG1* in different types of cancer. Disruption of *BRG1* was observed in several human cancer cell lines, including lung, breast, brain, colon, ovarian, and less frequently, pancreas¹⁸. In particular, the involvement of *BRG1* in lung cancers is well documented¹⁹⁻²².

Brg1 protein may play a role in the development of breast cancer, since it has been shown to promote growth suppression mediated by the estrogen receptor and BRCA1 interaction^{23;24}. Moreover, *BRG1* haploinsufficient mice were shown to develop mammary tumors²⁵.

Bi-allelic inactivating mutations in the *BRG1* gene have recently been reported in a minor subset (~2%) of pancreatic cancer, suggesting that *BRG1* may play a role in its development and progression²⁶.

Based upon this evidence, we immunolabeled a series of IPMNs for the Brg1 protein to determine if this protein may have a role in its development.

Methods

Patients and Tissues

The study was approved by the Johns Hopkins Institutional Review Board. For the analysis of Brg1 protein expression we used tissue microarrays (TMA) created from 66 independent archival surgically resected IPMNs²⁷. These 66 IPMNs are representative of the spectrum of IPMN subtypes and grades of dysplasia, and were classified according to the most recent WHO guidelines²⁸. Hence, with respect to the predominant characteristics of the epithelium, all IPMNs were defined as gastric-foveolar, pancreatobiliary, intestinal or oncocytic, and, based on the maximum cytoarchitectural atypia in the intraductal component, were graded as having low-, intermediate-, or high-grade dysplasia. The presence or absence of an associated invasive adenocarcinoma was also documented. Matched non-neoplastic pancreatic tissue cores were available for the IPMNs arrayed on the TMA.

Immunohistochemistry

Unstained 4µm sections were cut from each tissue microarray. Immunolabeling for the Brg1 protein was performed with a 1:100 dilution of murine monoclonal anti-BRG1 antibody (clone G-7, catalog #17796, Santa Cruz Biotechnology). Labeling was performed on a Leica-Bond autostainer using biotin free polymer detection reagents (Leica Microsystems). Briefly, after deparaffinization and hydration of the tissue microarray sections, antigen retrieval was achieved in EDTA buffer (pH 9.0) for 20 minutes. Tissues were incubated with the primary antibody for 15 minutes at room temperature, followed by incubation with postprimary and polymer detection for 8 minutes each. Chromogenic detection was performed using 3,3-diaminobenzidine and the slides counterstained with hematoxylin. Slides were then dehydrated and mounted permanently for evaluation.

Grading of labeling

Immunolabeling was evaluated by four of the authors (AM, MDM, SMH, HM) on a multi-headed microscope with consensus reached in all cases. Each tissue core was assessed independently without knowledge of the patient group. Based on the degree of nuclear labeling in the neoplastic epithelium, a three-tier intensity score (0: absent or minimal; 1: weak; 2: intense) was used for evaluation. In cases with heterogeneous labeling intensity, the respective tissue core was classified according to the predominant pattern of expression in the neoplastic cells. Neoplasms were given an area score from 0 to 4 based on the proportion of cells with labeling ("0": <5%, "1": 5–25%, "2": 26–50%, "3": 51–75%, "4": 76–100% of the cells labeled). The scores from different tissue cores of the same patient were averaged in order to obtain one case specific value. A total labeling score was calculated for each patient by multiplying intensity score and area score yielding values from 0 to 8. A total score > 3 was considered as positive whereas a total labeling score of ≤3 was regarded as negative. This cutoff was selected because the highest labeling observed in the normal pancreatic epithelium was a score of 4.

Cell culture

The derivation of low passage IPMN 1T cells has recently been described²⁹. The cells were maintained in MEM with 20% FBS (GIBCO), 5 ng/ml EGF (Promega), 0.2 U/ml human recombinant insulin (Invitrogen), and 1% penicillin streptomycin (Invitrogen). The hTERT immortalized Human Pancreatic Nestin Expressing epithelial cell line (HPNE cell line) was maintained according to established protocols³⁰. The human pancreatic cancer cell line Pa03C was maintained in DMEM containing 10% FBS and 1% Penicillin Streptomycin²⁶.

Western blot

Cells grown to confluence were scraped in RIPA buffer and sonicated at high power to lyse cells. 50 µg of protein lysate was resuspended in sample buffer, separated by SDS-PAGE and transferred to nitrocellulose membranes. The membrane was blocked in TBST containing 5% powdered milk for 30 min and incubated in anti-Brg1 antibody (H-88; Santacruz) for 1 h to overnight. Blots were probed with HRP-conjugated antirabbit secondary antibody (Santacruz). HRP activity was detected using western lighting chemiluminescence reagents (Pierce).

Statistical analysis

Statistical computations were performed using SPSS version 17 (SPSS Inc., Chicago, IL, USA). Comparison of categorical expression parameters were examined using Fisher's exact tests. Comparison of means was performed using Mann-Whitney test. A *P*-value of less than 0.05 was considered statistically significant.

Results

Brg1 protein expression decreases with increasing dysplasia in IPMN lesions

Of the 66 IPMN lesions 60 (90.1%) were suitable for evaluation of Brg1 immunohistochemical labeling. This included 14 (23.3%) lesions with low-grade dysplasia, 29 (48.3%) with intermediate-grade dysplasia, and 17 (28.3%) with high grade dysplasia. Representative examples for each labeling category are shown in Figure 1. Eight IPMNs presented with an associated invasive pancreatic ductal adenocarcinoma, all tubular subtype. Both the IPMN and the associated infiltrating cancer were available for analysis.

Matched normal pancreatic ductal epithelium was available for 51 IPMNs (83.3%) and all normal epithelial samples were positive for Brg1 nuclear expression. A full labeling score of 8 was seen in nearly all evaluable "normal" tissues (n=45; 88.2%) (Figure 2). In contrast, Brg1 labeling was reduced in 32 of the 60 (53.3%) IPMN lesions. This loss was observed significantly more often in high grade IPMN lesions (13 of 17; 76%) compared to low grade (4 of 14; 29%) and intermediate-grade IPMNs (15 of 29; 48%; *p*=0.03). A complete loss of expression was noted in 5 (8.3%) lesions comprising 4 IPMNs with intermediate-grade and 1 IPMN with high-grade dysplasia. We did not observe a statistical difference in Brg1 labeling scores among different histological IPMN subtypes. Furthermore, total labeling score did not correlate with tumor location within the ductal tree (main-duct vs. branch-duct vs. mixed) (Table 1). Brg1 labeling was negative in 2 out of 8 invasive cancers (25%). However, no significant difference was observed, when the mean labeling score of the cancers was compared with the mean value of their matched non-invasive IPMNs (Table 2).

To further substantiate our immunohistochemical findings in tissue sections, Western blot analysis was subsequently performed, to evaluate Brg1 expression in the low passage non-invasive IPMN cell line, IPMN 1T²⁹. As a positive control for a cancer with known somatic mutation of *BRG1*, we used the Pa03C line, as recently described in the pancreatic cancer genome sequencing effort²⁶. When compared with HPNE cell lines, both Pa03C and the IPMN 1T cells showed a marked and comparable decrease in Brg1 protein expression (Figure 3).

Discussion

Pancreatic cancer continues to be one of the most lethal gastrointestinal malignancies³¹. Currently, surgical resection of lesions detected at the earliest possible stage represents the greatest chance for cure. However, very few cancers are diagnosed at an early stage, and

known precursor lesions of pancreatic cancer, such as IPMN or Mucinous Cystic Neoplasms (MCNs) do not harbor the same malignant potential in every single case, rendering their appropriate management difficult to assess. Therefore, there is a great need to better characterize the molecular biology of cystic precursor lesions such as IPMNs, in order to better stratify the management for individual patient needs.

Recently, high throughput sequencing of various human cancers have uncovered recurrent mutations in genes involved in chromatin remodeling^{32;33}. Vogelstein B. et al., have demonstrated that mutations of *MEN1*, which encodes the histone methyltransferase component menin, occur at a frequency of 44% in pancreatic neuroendocrine tumors (PanNETs). Another 43% of PanNETs harbor mutations in *DAXX* (death-domain-associated protein) and *ATRX* (a thalassemia/mental retardation syndrome X-linked) whose protein products are also involved in chromatin remodeling³⁴.

Brg1 is an ATPase/helicase and as such constitutes the catalytic subunit of the SWI/SNF chromatin remodeling complex. This complex disrupts the adhesion of histone components to DNA thereby giving transcription factors access to their target genes^{35;36}. In mice, developmental homozygous deletion of *BRG1* results in peri-implantation lethality, whereas *BRG1* heterozygotes are predisposed to exencephaly and apocrine like tumors³⁷.

Abnormalities in the expression of *BRG1* have been reported in a number of different cancer types such as lung, breast, brain, colon, ovarian, and less frequently, pancreas^{18;20;21;23;24;38}. Functional studies have reiterated the putative tumor suppressive effects of Brg1 protein in human cancer cells. For example, knock-in of functional *BRG1* in a breast cancer cell line with a mutant *BRG1* allele induces growth inhibition. This inhibition appears to be at least partly mediated by the suppression of cyclin E as an E2F target gene, and the overexpression of transcripts of cyclin-dependent kinase inhibitors p21 and p15³⁶. It has also been shown that Brg1 itself interacts with tumor suppressor proteins (e.g. RB1, BRCA1) and components involved in Wnt signaling^{24;39-45}, suggesting a role for this gene not only in chromatin remodeling, but also in cell-cycle regulation and in the activity of tumor-suppressor factors.

The role of *BRG1* in pancreatic carcinogenesis is poorly defined. The recent sequencing of the pancreatic cancer genome elucidated that somatic mutations of *BRG1* occur in ~2% of pancreatic cancers²⁶. Rosson et al., found that reduction of *BRG1* expression in pancreatic cancer cell lines by lentiviral short hairpin shRNA mediated RNA interference resulted in morphologic changes; however, no significant impact on *in vitro* growth of cultured cell lines could be observed⁴⁶.

In this study, we investigated the putative role of *BRG1* in IPMNs, the most common cystic precursor lesion of pancreatic cancer, by performing immunohistochemical evaluation of BRG1 expression on IPMN tissue microarrays.

We found a progressive loss of Brg1 expression associated with increasing degrees of dysplasia in IPMNs. Only 28% of low-grade IPMNs showed loss of Brg1 expression, compared to 76% of high-grade lesions ($p=0.03$) (Table 1). Intermediate-grade IPMNs displayed an intermediate percentage of loss (52%), suggesting a progressive decrease of expression from low- towards high-grade lesions. Loss of Brg1 expression was found in 2 out of 8 invasive cancer present in our TMA (25%) and this pattern was highly concordant with their non-invasive counterpart. This finding substantiates the progressive loss of Brg1 during tumor progression from lesions with mild dysplasia to invasive cancer. Interestingly we did not observe a difference in Brg1 expression among different histologic subtypes nor among different locations within the ductal system. It is known that main duct IPMNs are more likely to exhibit pancreaticobiliary or intestinal features and high-grade dysplasia and

are more commonly associated with an invasive carcinoma compared to branch duct lesions. However, loss of Brg1 does not appear to be involved in a phenotype differentiation nor to a preferential location within the ductal tree.

In order to extrapolate the tissue studies to cultured cell lines, especially in a recently described *in vitro* IPMN model ²⁹, we assessed Brg1 expression in IPMN and PDAC cells. When compared with non-neoplastic HPNE cells, IPMN 1T showed a marked decrease in Brg1 expression, which was comparable to the “positive” control Pa03C cells, that are known to harbor a somatic mutation of *BRG1*. This finding corroborates our results and provides additional support to the role of Brg1 in IPMN development and progression. Further, the Pa03C and IPMN 1T cells provide a novel *in vitro* platform to study the functional consequences of *BRG1* restitution in pancreatic neoplasia for the first time.

Conclusion

Our study shows that a progressive loss of immunolabeling for the Brg1 protein, confirmed by Western blot analysis on cell lines, is associated with increasing degrees of dysplasia in IPMNs. These results suggest an involvement in tumor progression and therefore contribute to the growing body of literature focusing on chromatin remodeling complexes and their role in cancer formation.

Further biological and biochemical analyses are needed to better characterize and validate the biological significance of Brg1 in pancreatic carcinogenesis.

Acknowledgments

This work was supported by R01CA113669, P50CA62924, P01CA134292, the Sol Goldman Pancreatic Cancer Research Center and the Michael Rolfe Foundation for Pancreatic Cancer Research. Hanno Matthaei is supported by a grant from the Mildred-Scheel-Stiftung, Deutsche Krebshilfe, Bonn, Germany.

References

1. Fernandez-del Castillo C, Targarona J, Thayer SP, Rattner DW, Brugge WR, Warshaw AL. Incidental pancreatic cysts: clinicopathologic characteristics and comparison with symptomatic patients. *Arch Surg.* 2003; 138(4):427–3. discussion 33–4. [PubMed: 12686529]
2. Brugge WR, Lewandrowski K, Lee-Lewandrowski E, et al. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. *Gastroenterology.* 2004; 126(5):1330–6. [PubMed: 15131794]
3. Spinelli KS, Fromwiller TE, Daniel RA, et al. Cystic pancreatic neoplasms: observe or operate. *Ann Surg.* 2004; 239(5):651–7. discussion 57–9. [PubMed: 15082969]
4. Winter JM, Cameron JL, Lillemoe KD, et al. Periampullary and pancreatic incidentaloma: a single institution's experience with an increasingly common diagnosis. *Ann Surg.* 2006; 243(5):673–80. [PubMed: 16633003]
5. Hruban RH, Maitra A, Kern SE, et al. Precursors to pancreatic cancer. *Gastroenterol Clin North Am.* 2007; 36:831–849. [PubMed: 17996793]
6. Schönleben F, et al. BRAF and KRAS gene mutations in intraductal papillary mucinous neoplasm/ carcinoma (IPMN/IPMC) of the pancreas. *Cancer Lett.* 2007; 249:242–248. [PubMed: 17097223]
7. Abe K, Suda K, Arakawa A, Yamasaki S, Sonoue H, Mitani K, Nobukawa B. Different patterns of p16INK4A and p53 protein expressions in intraductal papillary-mucinous neoplasms and pancreatic intraepithelial neoplasia. *Pancreas.* 2007 Jan; 34(1):85–91. [PubMed: 17198188]
8. Sasaki S, Yamamoto H, Kaneto H, Ozeki I, Adachi Y, Takagi H, Matsumoto T, Itoh H, Nagakawa T, Miyakawa H, Muraoka S, Fujinaga A, Suga T, Satoh M, Itoh F, Endo T, Imai K. Differential roles of alterations of p53, p16, and SMAD4 expression in the progression of intraductal papillary-mucinous tumors of the pancreas. *Oncol Rep.* 2003 Jan-Feb; 10(1):21–5. [PubMed: 12469138]

9. Iacobuzio-Donahue CA, Klimstra DS, Adsay NV, Wilentz RE, Argani P, Sohn TA, Yeo CJ, Cameron JL, Kern SE, Hruban RH. Dpc-4 protein is expressed in virtually all human intraductal papillary mucinous neoplasms of the pancreas: comparison with conventional ductal adenocarcinomas. *Am J Pathol.* 2000; 157:755–761. [PubMed: 10980115]
10. Sato N, Rosty C, Jansen M, Fukushima N, Ueki T, Yeo CJ, Cameron JL, Iacobuzio-Donahue CA, Hruban RH, Goggins M. STK11/LKB1 Peutz-Jeghers gene inactivation in intraductal papillary-mucinous neoplasms of the pancreas. *Am J Pathol.* 2001; 159:2017–2122. [PubMed: 11733352]
11. Schonleben F, Qiu W, Ciau NT, et al. PIK3CA mutations in intraductal papillary mucinous neoplasm/carcinoma of the pancreas. *Clin Cancer Res.* 2006; 12:3851–3855. [PubMed: 16778113]
12. Sato N, Fukushima N, Maitra A, Iacobuzio-Donahue CA, van Heek NT, Cameron JL, Yeo CJ, Hruban RH, Goggins M. Gene expression profiling identifies genes associated with invasive intraductal papillary mucinous neoplasms of the pancreas. *Am J Pathol.* 2004; 164:903–914. [PubMed: 14982844]
13. Abe T, Fukushima N, Brune K, Boehm C, Sato N, Matsubayashi H, Canto M, Petersen GM, Hruban RH, Goggins M. Genome-wide allelotypes of familial pancreatic adenocarcinomas and familial and sporadic intraductal papillary mucinous neoplasms. *Clin Cancer Res.* 2007; 13:6019–6025. [PubMed: 17947463]
14. Fritz S, Fernandez-del Castillo C, Mino-Kenudson M, Crippa S, Deshpande V, Lauwers GY, Warshaw AL, Thayer SP, Iafraite AJ. Global genomic analysis of intraductal papillary mucinous neoplasms of the pancreas reveals significant molecular differences compared to ductal adenocarcinoma. *Ann Surg.* 2009; 249:440–447. [PubMed: 19247032]
15. Soldini D, Gugger M, Burckhardt E, Kappeler A, Laissue JA, Mazzucchelli L. Progressive genomic alterations in intraductal papillary mucinous tumours of the pancreas and morphologically similar lesions of the pancreatic ducts. *J Pathol.* 2003; 199:453–461. [PubMed: 12635136]
16. Schlegel C, Arens N, Zentgraf H, Bleyl U, Verbeke C. Identification of frequent chromosomal aberrations in ductal adenocarcinoma of the pancreas by comparative genomic hybridization (CGH). *J Pathol.* 2000; 191:27–32. [PubMed: 10767715]
17. Wolffe AP. Chromatin remodeling: why it is important in cancer. *Oncogene.* 2001; 20(24):2988–90. [PubMed: 11420713]
18. Wong AK, Shanahan F, Chen Y, et al. BRG1, a component of the SWI-SNF complex, is mutated in multiple human tumor cell lines. *Cancer Res.* 2000; 60(21):6171–7. [PubMed: 11085541]
19. Glaros S, Cirrincione GM, Palanca A, Metzger D, Reisman D. Targeted knockout of BRG1 potentiates lung cancer development. *Cancer Res.* 2008 May 15; 68(10):3689–96. [PubMed: 18483251]
20. Fukuoka J, Fujii T, Shih JH, et al. Chromatin remodeling factors and BRM/BRG1 expression as prognostic indicators in non-small cell lung cancer. *Clin Cancer Res.* 2004; 10(13):4314–24. [PubMed: 15240517]
21. Reisman DN, Sciarrotta J, Wang W, Funkhouser WK, Weissman BE. Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: correlation with poor prognosis. *Cancer Res.* 2003; 63(3):560–6. [PubMed: 12566296]
22. Rodriguez-Nieto S, Cañada A, Pros E, Pinto AI, Torres-Lanzas J, Lopez-Rios F, Sanchez-Verde L, Pisano DG, Sanchez-Cespedes M. Massive parallel DNA pyrosequencing analysis of the tumor suppressor BRG1/SMARCA4 in lung primary tumors. *Hum Mutat.* 2011 Feb; 32(2):E1999–2017. [PubMed: 21280140]
23. Wang S, Zhang B, Faller DV. BRG1/BRM and prohibitin are required for growth suppression by estrogen antagonists. *EMBO J.* 2004; 23(11):2293–303. [PubMed: 15141164]
24. Bochar DA, Wang L, Beniya H, et al. BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell.* 2000; 102(2):257–65. [PubMed: 10943845]
25. Bultman SJ, Herschkowitz JI, Godfrey V, et al. Characterization of mammary tumors from Brg1 heterozygous mice. *Oncogene.* 2008; 27(4):460–8. [PubMed: 17637742]
26. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science.* 2008; 321(5897):1801–6. [PubMed: 18772397]

27. Hong SM, Kelly D, Griffith M, et al. Multiple genes are hypermethylated in intraductal papillary mucinous neoplasms of the pancreas. *Mod Pathol*. 2008; 21(12):1499–507. [PubMed: 18820670]
28. Bosman, FCF.; Hruban, R.; Theise, N. WHO Classification of Tumours of the Digestive System IARC. Lyon: 2010.
29. Kamiyama H, Kamiyama M, Hong SM, Karikari CA, Lin MT, Borges MW, Griffith M, Young A, Norris-Kirby A, Lubek C, Mizuma M, Feldmann G, Shi C, Liang H, Goggins MG, Maitra A, Hruban RH, Eshleman JR. In vivo and in vitro propagation of intraductal papillary mucinous neoplasms. *Lab Invest*. 2010 May; 90(5):665–73. [PubMed: 20231822]
30. Lee KM, Nguyen C, Ulrich AB, Pour PM, Ouellette MM. immortalization with telomerase of the Nestin-positive cells of the human pancreas. *Biochem Biophys Res Commun*. 2003 Feb 21; 301(4):1038–44. [PubMed: 12589817]
31. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011
32. Muchardt C, Yaniv M. When the SWI/SNF complex remodels...the cell cycle. *Oncogene*. 2001; 20(24):3067–75. [PubMed: 11420722]
33. Wolffe AP. Chromatin remodeling: why it is important in cancer. *Oncogene*. 2001; 20(24):2988–90. [PubMed: 11420713]
34. Jiao Y, Shi C, Edil BH, et al. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science*. 2011; 331(6021):1199–203. [PubMed: 21252315]
35. Reisman D, Glaros S, Thompson EA. The SWI/SNF complex and cancer. *Oncogene*. 2009; 28(14):1653–68. [PubMed: 19234488]
36. Hendricks KB, Shanahan F, Lees E. Role for in cell cycle control and tumor suppression. *Mol Cell Biol*. 2004; 24(1):362–76. [PubMed: 14673169]
37. Bultman S, Gebuhr T, Yee D, et al. A BRG1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Mol Cell*. 2000; 6(6):1287–95. [PubMed: 11163203]
38. Medina PP, Carretero J, Fraga MF, Esteller M, Sidransky D, Sanchez-Cespedes M. Genetic and epigenetic screening for gene alterations of the chromatin-remodeling factor, SMARCA4/BRG1, in lung tumors. *Genes Chromosomes Cancer*. 2004; 41(2):170–7. [PubMed: 15287030]
39. Roberts CW, Orkin SH. The SWI/SNF complex--chromatin and cancer. *Nat Rev Cancer*. 2004; 4(2):133–42. [PubMed: 14964309]
40. Klochendler-Yeivin A, Muchardt C, Yaniv M. SWI/SNF chromatin remodeling and cancer. *Curr Opin Genet Dev*. 2002; 12(1):73–9. [PubMed: 11790558]
41. Barker N, Hurlstone A, Musisi H, Miles A, Bienz M, Clevers H. The chromatin remodelling factor Brg-1 interacts with beta-catenin to promote target gene activation. *EMBO J*. 2001; 20(17):4935–43. [PubMed: 11532957]
42. Eroglu B, Wang G, Tu N, Sun X, Mivechi NF. Critical role of Brg1 member of the SWI/SNF chromatin remodeling complex during neurogenesis and neural crest induction in zebrafish. *Dev Dyn*. 2006; 235(10):2722–35. [PubMed: 16894598]
43. Murphy DJ, Hardy S, Engel DA. Human SWI-SNF component BRG1 represses transcription of the c-fos gene. *Mol Cell Biol*. 1999; 19(4):2724–33. [PubMed: 10082538]
44. Pal S, Yun R, Datta A, et al. mSin3A/histone deacetylase 2- and PRMT5-containing Brg1 complex is involved in transcriptional repression of the Myc target gene cad. *Mol Cell Biol*. 2003; 23(21):7475–87. [PubMed: 14559996]
45. Decristofaro MF, Betz BL, Rorie CJ, Reisman DN, Wang W, Weissman BE. Characterization of SWI/SNF protein expression in human breast cancer cell lines and other malignancies. *J Cell Physiol*. 2001; 186(1):136–45. [PubMed: 11147808]
46. Rosson GB, Bartlett C, Reed W, Weissman BE. BRG1 loss in MiaPaCa2 cells induces an altered cellular morphology and disruption in the organization of the actin cytoskeleton. *J Cell Physiol*. 2005; 205(2):286–94. [PubMed: 15887247]

Figure 1.

Based on the degree of labeling in the neoplastic epithelium, a three-tier intensity score was used for evaluation. Representative examples are shown (Panel A: 0 = absent or minimal labeling; Panel B: 1 = weak labeling; Panel C: 2 = intense labeling).

Figure 2.

Panel A) Intact Brg1 labeling in a normal pancreatic epithelium. Panel B) Loss of Brg1 labeling in a high-grade IPMN lesion. Note retained expression in subjacent normal pancreatic ductal epithelium. Panel C) Loss of Brg1 labeling in cancer arising from a high-grade IPMN lesion.

Figure 3. Western Blot analysis shows a markedly decreased Brg1 expression in IPMN 1T cell line, compared with HPNE and Pa03C cells.

Brg-1 Expression of IPMNs diverges significantly with respect to grade of dysplasia (IPMN low-grade and intermediate grade vs. IPMN with high-grade dysplasia, $p=0.03$).

Table 1

		Brg1-Expression			P-value
		Minimal or absent	Present	Total	
Histologic grade	Low-grade	4, (28%)	10 (72%)	14	0.03*
	Intermediate-grade	15 (52%)	14 (48%)	29	
	High-grade	13 (76%)	4 (24%)	17	
Histologic type	Pancreatobiliary	18 (50%)	18 (50%)	36	0.27
	Intestinal	9 (43%)	7 (57%)	16	
	Gastric	5 (83%)	1 (17%)	6	
	Oncocytic	0	1	1	
	Unclassified	0	1	1	
Location	Main duct	16 (48%)	17 (52%)	33	0.13
	Branch duct	10 (59%)	7 (41%)	17	
	Combined	4 (67%)	2 (33%)	6	
	Unknown	2 (50%)	2 (50%)	4	

Table 2

Labeling score of 8 invasive cancers and their correspondent high-grade IPMN. No significant difference is appreciated between the two groups, regarding the loss of Brg1 expression ($p= 0.655$).

Case n°	Brg1 Total Labeling score		P-value
	Invasive Cancer	IPMN	
1	4	6	0.655
2	8	6	
3	1	3	
4	8	6	
5	8	6	
6	8	8	
7	4	4	
8	0	0	