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Distinct Genome-Wide Methylation Patterns in Sporadic and Hereditary Nonfunctioning Pancreatic Neuroendocrine Tumors

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Abstract

Background—Aberrant methylation is a known cause of cancer initiation and/or progression. There is scant data on the genome-wide methylation pattern of non-functioning pancreatic neuroendocrine tumors (NFPanNETs) and in sporadic and hereditary NFPanNETs.

Methods—Thirty-three tissue samples were analyzed, including sporadic (n=9), von Hippel-Lindau [VHL]-related and multiple endocrine neoplasia type 1 [MEN1]-related NFPanNETs (n=10 each), which were compared to normal islet cells (NI, n=4). Genome-wide CpG methylation profiling was performed using the Infinium MethylationEPIC BeadChips Assay and analyzed by R-based tools.

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The authors declare that they have nothing to disclose.

Results—On unsupervised hierarchical clustering, sporadic and MEN1-related NFPanNETs clustered together, and the VHL group in a separate cluster. MEN1-related NFPanNET had a higher rate of hypermethylated CpG sites comparing to sporadic and VHL-related tumor groups. Differentially methylated region (DMR) analysis confirmed the higher rate of hypermethylation in MEN1-related tumors. Moreover, in integrated analysis of gene expression data in the same tumor samples, we found downregulated gene expression in most genes that were hypermethylated. In a CpG Island Methylator Phenotype (CIMP) analysis, three genes were identified and confirmed to have downregulated gene expression, *SFRP5* in sporadic NFPanNET, and *CDCA7L* and *RBM47* in MEN1-related NFPanNET.

Conclusions—MEN1 NFPanNETs have a higher rate of genome-wide hypermethylation compared with other NFPanNET subtypes. The similarity between pathways enriched on methylation analysis of known genes involved in NFPanNET tumorigenesis suggest a key role for aberrant methylation in the pathogenesis of NFPanNETs.

Precis

There is scant data on the genome-wide methylation pattern of sporadic and hereditary non-functioning pancreatic neuroendocrine tumors (NFPanNETs).

In the current analysis we compared genome-wide methylome of NFPanNETs of patients with and without hereditary syndromes, and found a higher rate of hypermethylation in those related to multiple endocrine neoplasia type 1, and hypomethylation in von Hippel-Lindau-related tumors.

Keywords

pancreatic neuroendocrine tumor; DNA methylation; MEN-1; VHL; nonfunctioning

Introduction

The incidence of pancreatic neuroendocrine tumors (PanNETs) has been increasing in the last decades, currently with an incidence of 0.48 cases per 100,000 inhabitants annually in the United States (1–3). PanNETs are categorized as functioning (secreting compounds that lead to a clinical syndrome) and nonfunctioning tumors (NFPanNET), the latter being more prevalent. Functional PanNETs, even when localized and small, require treatment to control the clinical manifestations, and morbidity and mortality caused by the excess hormone production. In contrast, small, low-grade NFPanNETs do not always require surgical or medical intervention, as they may have an indolent clinical course, but the natural history of such tumors is heterogenous and unpredictable. The current recommendations for treatment of syndromic NFPanNETs are mostly based on the risk of metastatic disease, using tumor growth and size as a surrogate marker for aggressiveness [1].

PanNETs can occur sporadically or in the context of an inherited cancer syndrome. These include Multiple Endocrine Neoplasia Type 1 (MEN1), von Hippel-Lindau (VHL), Neurofibromatosis type 1, Tuberous Sclerosis Complex and Cowden syndrome [1–4]. About 40% of patients with MEN1 [5], and about 15% of patients with VHL [1] develop NFPanNETs during their lifetime. Syndromic PanNETs may have a less aggressive natural history than their sporadic counterparts [6–8]. Hence the criteria used for sporadic NFPanNETs risk

stratification do not accurately predict the metastatic potential and/or aggressive behavior of NFPanNETs in patients with VHL and MEN1 [9–13]. The biologic mechanism underlying this difference is not well understood [1, 14].

The genes responsible for the major inherited cancer syndromes associated with NFPanNETs (VHL, MEN1) have been well characterized. In VHL, a broad genotype-phenotype association was demonstrated for PanNET, with missense and/or exon 3 pathogenic variants associated with high risk of disease progression and metastasis [6, 15]. In contrast, no clear genotype-phenotype association has been found in MEN1. Also, a growing number of studies have characterized the main genes and molecular pathways associated with the tumorigenesis of sporadic PanNETs [16, 17], and reported a high rate of somatic mutations in *MEN1, DAXX, ATRX,* and *PTEN*.

The best characterized epigenetic event involved in cancer initiation and/or progression is DNA methylation of cytosines, by DNA methyltransferases. The dinucleotide sequence of cytosine followed by guanine is termed CpG, and a high-density cluster of CpGs are referred to as CpG island. Approximately 60% of CpG islands are in the 5’ regulatory (promoter) regions of genes, and have key role in transcription regulation, although CpG islands that are not in promoter regions may also be targets for *de novo* methylation in cancer. Furthermore, differential CpG methylation in DNA enhancer regions have also been implicated in cancer and altered gene expression [18]. DNA methylation affects a wide range of molecular and cellular processes involved in cancer initiation and progression, such as apoptosis, cell cycle, DNA damage repair, growth factor response, signal transduction, and more [19].

Gene-specific CpG promoter hypermethylation has been reported in a variety of NETs including the pancreas, pituitary, and adrenal glands. The genes with dysregulated methylation included *CDKN2A*, *CDKN2B*, *APC*, *CTNNB1*, *HIC1*, *RIZ1, MEG3*, *MLH1* and *RASSF1A* [20–23]. Furthermore, specific methylation analysis has shown a high rate of promoter hypermethylation in MEN1-related tumors in an analysis of 56 genes [24]. To our knowledge, the genome-wide methylation profiling of NFPanNETs has not been reported, both sporadic and syndromic, as well as, an integrated analysis of methylation and gene expression. Furthermore, the epigenetically regulated pathway(s) involved in NFPanNETs are unknown. Such data could be useful for better understanding the pathobiology of NFPanNETs and for identifying new markers of disease behavior, and markers for diagnosis of NFPanNETs, and can help identify new targets for treatment of both syndromic and sporadic NFPanNETs with epigenetics modulating drugs [25, 26].

Given the limited data on the importance of methylation in NET and the lack of genome-wide methylome data of inherited and sporadic NFPanNETs, we investigated the genome-wide methylome of sporadic and syndromic (MEN1 and VHL) NFPanNETs with integrated gene expression data in the same samples.

Materials and Methods

Patients

The study protocol was approved by the institutional review board, and written informed consent was obtained from all patients. Patients were diagnosed with VHL or MEN1 based on either germline DNA analysis revealing a pathogenic variant, or according to established clinical criteria for VHL [15] and MEN1 [27]. Patients clinical data were retrieved from their medical records, and the tumor (non) functional status was determined by clinical features, and by biochemical testing as clinically indicated. Tissue samples included 9 sporadic NFPanNET, 10 MEN1 NFPanNET, 10 VHL NFPanNET and 4 NI samples. Detailed clinical and histopathologic data of the study cohort are summarized in Table 1.

Tissue procurement

Tissue samples were obtained at the time of surgical resection. Samples were snap frozen and immediately stored at −80°C. Tissue sections adjacent to those used for RNA and DNA isolation were stained using hematoxylin-eosin to confirm the diagnosis and a tumor cell content >80%. All tissues included in the study underwent secondary histologic review by an endocrine pathologist (MMQ).

Normal islet cell samples

Normal islet cell (NI) samples were obtained from the Division of Transplantation, Department of Surgery at the University of Alabama at Birmingham. Pancreas were recovered, with informed consent, from cadaveric donors after *in situ* vascular perfusion with University of Wisconsin solution at 4°C, as part of a multiorgan procurement. The pancreatic islets were isolated by a semiautomated method and purified using the Cobe 2991 cell processor (Gambro BCT, Lakewood, CO). The number of islets within each size class was converted to the standard number of islets of 150-μm diameter, equal in volume to the sample. Purity was assessed by comparing the relative quantity of dithizone-stained endocrine tissue with unstained exocrine tissue. Only islets with greater than 90% viability and greater than 60% purity were used.

DNA extraction and processing

Frozen NFPanNET tissue samples were sectioned and DNA was extracted using the DNeasy blood and tissue kit (QIAGEN, Valencia, CA) according to the manufacturer protocol. DNA quality was determined using the NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE) and quantified using the Quant-iT PicoGreen dsDNA Assay (Life Technologies, Grand Island NY).

Gene expression analysis

Gene expression analysis was performed as described previously [28]. Briefly, sample labeling, and array hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technologies). Total RNA from each sample was linearly amplified and labeled. The labeled complementary RNAs (cRNAs) were purified using the RNeasy Mini Kit (Qiagen), and the labeled cRNAs were

measured using the NanoDrop ND-1000 spectrophotometer. One microgram of each labeled cRNA was fragmented, hybridized and scanned using the Agilent DNA Microarray Scanner (Agilent Technologies).

Methylation data processing and analysis

Methylation analysis and data quality control were performed on R version 3.4.4 and R Studio Version 1.0.153, using the minfi package [29], which was modified for the analysis of Illumina EPIC arrays [30]. All comparisons were based on the M values calculated by the minfi package from the corresponding beta values. The minfi package was also used for functional normalization [31], and for differentially methylated probe (DMP, at each CpG site) detection.

Regions with higher and lower M values compared to NI values were considered hyper- and hypomethylated, respectively. DMPs with at least 2-fold change with a false discovery rate adjusted p-value < 0.05 were considered significantly different and were included for further analysis. Pathway analysis using DMPs was performed using the ReacromePA package [32].

Differentially methylated regions (DMRs) were detected using the bumphunter package [33], based on a maximal gap of 300, M value ratio of 0.55 and 500 permutations. Significantly different regions were considered based on an area family-wise error rate (FWER) <0.05. CpG island methylator phenotype (CIMP) analysis was done to detect genes with a statistically significant hypermethylation in gene promoter regions compared to NI samples based on the DMR analysis results. Promoter regions were annotated using the matchGenes function of bumphunter package, with default parameters.

To confirm that our results were not biased by NI impurity, we analyzed the methylation status of different NFPanNETs based the raw M values. We compared the rate of CpG hypomethylation and hypermethylation (mean M-value < −2 or > 2, respectively). Methylation levels were compared using the analysis of variances (ANOVA) with the Tukey adjustment method (Supplementary Figure 1).

To assess the full extent of the implications of altered methylation on gene expression, we performed an ENCODE based analysis for differential methylation in gene enhancers. All DMRs were compared with the RegulomeDB (http://www.regulomedb.org). We considered coordinates as enhancers if they were verified by expression quantitative trait loci (RegulomoeDB score of 1f or above). The enhancers detected were further verified by up- and down-regulation of gene expression in hypo- and hypermethylated gene enhancers, respectively.

The heatmap and unsupervised hierarchical clustering analysis presented in Figure 1 were analyzed using the Partek Genomic suite 7.17 software (Partek Inc., St. Louis, MO).

Results

Different methylation pattern between subgroups of NFPanNETs

A total of 15,844 CpG sites were found to be differentially methylated between NFPanNETs and NIs. Unsupervised clustering analysis, using the top 5000 DMPs, showed clustering of the VHL NFPanNETs together and a separate cluster of sporadic and MEN1 NFPanNETs (Figure 1A). A total of 4666, 2780 and 25633 DMPs in the sporadic, VHL and MEN1 groups, respectively, were found to be significantly different with a delta M value > 2 (hypermethylated) or < −2 (hypomethylated) compared to NI samples. The rate of hypomethylated CpG sites was significantly higher among VHL-related NFPanNET (66.3%) compared with both sporadic (33.7%) and MEN-1-related NFPanNET (37.8%, $p<0.001$ for both comparisons) (Figure 1B). We further analyzed the methylation patterns by overall cancer stage including tumor size and lymph node metastasis, and tumor grade, and found no statistically significant DMPs.

Pathway analysis

We performed pathway analysis based on the genes annotated in the DMP positions. In sporadic NFPanNETs, the main pathways enriched included the transcription regulator RUNX3, PLC-related pathway, DAG/IP3 signaling, regulation of insulin secretion, and calcium-related pathways. In VHL-related tumors, among the most enriched pathways were intracellular signal transduction pathways, VEGF-related pathway, regulation of beta−cell development and RUNX1 expression and activity. Whereas in MEN1-related tumors, neuronal pathways, VEGF signaling, insulin secretion regulation, calcium dependent pathway, G alpha and pophatidylinositol-4,5-bisphosphate (PIP2)-related pathway were enriched according to the aberrantly methylated genes (Figure 2).

Differentially methylated regions (DMRs)

We performed a DMR analysis to define key genomic regions more robustly. DMR analysis detected 34 DMRs in sporadic (18/16 hyper-/hypomethylated), 32 DMRs in VHL-related (9/23 hyper-/hypomethylated) and 66 DMRs in MEN1-related NFPanNETs (56/10 hyper-/hypomethylated), as compared to NI samples (Figure 3A). The total number of genes annotated for these regions were 34 in the sporadic group, 26 in the VHL group and 66 in the MEN1 group. Of them, 16 (8/8), 13 (4/9) and 29 (26/3) were associated with significantly differential gene expression compared with NI samples (Figure 3B). We found 5, 1 and 19 in 8, 4 and 26 hypermethylated genes in the sporadic, VHL and MEN1 groups, respectively, associated with downregulated gene expression, and 7, 7 and none of the 8, 9 and 3 hypomethylated genes were associated with upregulated gene expression, respectively (Figure 3B).

Candidate genes for CpG island methylator phenotype (CIMP)

To characterize a possible CIMP in NFPanNET, we compared methylation levels between each group and NI samples, and identified several genes with significantly hypermethylated promoter regions [34]. The analysis was based on the DMR analysis results and detected eight genes with promoter hypermethylation in the MEN1 group, two in the sporadic group and

none in the VHL group. To validate these findings, we evaluated gene expression data in the same samples as used for the methylation analysis. We found downregulated gene expression (based on a fold change <1.5 compared with NI samples, with a p-value < 0.05 of an unpaired t-test) in one of the two genes with promoter hypermethylation in the sporadic group (*SFRP5*) and 2 of the 8 genes in the MEN1 group (*CDCA7L* and *RBM47*) (Figure 4).

Aberrant methylation of enhancer regions

Significantly different methylation levels between NFPanNET and NI samples were detected in four gene enhancer regions (Table 2). The gene enhancer for *PTPRN2* was found to be significantly hypomethylated based on the DMR analysis in all three groups (sporadic, MEN1 and VHL), and was associated with upregulated *PTPRN2* expression in both sporadic (fold change of 12.7, p-value < 0.001) and MEN1 (fold change of 3.7, p-value = 0.003) NFPanNETs. In addition, hypomethylation of the enhancer for *TRPV2* was found in the VHL group, with upregulated gene expression (fold change of 4.7, p-value < 0.001). Enhancer hypermethylation was found in two genes (*SLC1A5*, *PQLC2*) in MEN1-related NFPanNETs, *SLC1A5*, was associated with downregulated gene expression (fold change of 3.0, p-value = 0.004).

Discussion

In this study, we analyzed the genome-wide methylation pattern of NFPanNETs, aiming to characterize aberrant methylation in these tumors, and possible differences between sporadic vs. hereditary NFPanNETs. We have shown that in MEN1 NFPanNETs, there are significantly more hypermethylated genomic positions as compared to sporadic and VHL NFPanNETs. In pathway analysis based on genes with an aberrant methylation patterns, pathways associated with the specific signaling pathways, such as VEGF in VHL, were identified. Finally, we also found three genes in which significant CpG promoter hypermethylation was detected, with downregulated gene expression. We identified altered methylation in several gene enhancers that was associated with altered gene expression in NFPanNETs.

The gene responsible for the MEN1 syndrome, *MEN1*, is located on chromosome 11q13 [35]. Germline *MEN1* mutations are detected in 70–90% of patients with a familial presentation of MEN1 [36]. Menin, the protein encoded by *MEN1*, participates in epigenetic control through direct interaction with protein-complexes and through regulation of non-coding RNAs. Altered menin function leads to epigenetic aberrations and consequently to tumorigenesis, possibly explaining the weak genotype-phenotype association based on *MEN1* mutation analysis [20]. Our findings of a high rate of methylation aberrations in MEN-1-related tumors compared with sporadic and VHL-related tumors further support the role of menin in the regulation of DNA methylation.

The results of the pathway analysis emphasize the possible key role of methylation in the pathogenesis of NFPanNETs. In all three groups, pathways found to be enriched are associated with NET pathogenesis and/or with islet cell function. These include insulin secretion regulation in sporadic and MEN1-related groups, and beta cell differentiation in the VHL group. The two transcription factors RUNX1 [37] and RUNX3 [38], enriched in the

VHL and sporadic groups, respectively, were previously reported to be altered in NET. Both PIP2 [39] and DAG/IP3 [40], are involved in the PI3K/AKT/mTOR pathway, which was reported by Scarpa and associates as one of the four main pathways of NFPanNET pathogenesis [17]. While Scarpa et al. showed that mutations in genes involved in these pathways are common, we now show that epigenetic alterations of genes involved in these pathways may be an alternative mechanism for dysregulation of these same pathways in NFPanNETs.

We identified three candidate genes for CIMP, based on promoter hypermethylation and downregulated gene expression. *SFRP5* (secreted frizzle-related protein 5), that was detected in sporadic NFPanNET in our analysis is associated with Wnt/ß-catenin signaling. Altered methylation of *SFRP5* was reported in esophageal squamous-cell carcinoma [41] and in treatment-resistance leukemia [42]. *SFRP5* has also been reported to be associated with beta cell function [43]. Promoter hypermethylation was detected in MEN1-related NFPanNETs in two genes: *CDCA7L* and *RBM47*. The former encodes the cell division cycle-associated 7-like protein, which interacts with c-Myc, a pathway altered regulated by menin [44]. *CDCA7L* was associated with hepatocellular carcinoma, and was found to have altered methylation and gene expression in pediatric pineal tumors [45]. The tumor-suppressive role of *RBM47* (RNA binding motif 47) was reported in colorectal and breast cancer [46, 47], and in lung adenocarcinomas [48]. Validation of the aberrant methylation of these genes' promoter region in an independent cohort in the future will be important to define CIMP in NFPanNET subtypes.

We have identified altered methylation in several gene enhancers. Among them the enhancer for the *PTPRN2* gene was found hypomethylated in all three groups. The gene *PTPRN2* (protein tyrosine phosphatase, receptor type N2) encodes the transmembrane protein PTP IA-2beta, which is highly expressed in the pancreatic islets, and known as a target antigen in autoimmune diabetes mellitus [49]. Altered methylation of *PTPRN2* has been reported in hepatocellular carcinoma [50] and lung cancer [51], but *PTPRN2* enhancer hypomethylation has not been reported in NETs. Enhancer methylation status has been implicated in cancer initiation [18], in defining subtypes of breast cancer [52], and in cancer plasticity and cancer survival [53]. However, to the best of our knowledge this is the first report evaluating enhancer methylation status and gene expression in NETs. The common hypomethylation of *PTPRN2* enhancer in all three groups of NFPanNET suggests this as a possible common pathogenic pathway in these tumors.

We have shown that aberrant methylation was associated with altered gene expression and thus likely to play an important role in NFPanNET initiation and or progression even in the presence of driver mutations. Moreover, the overlap in pathways between the different groups in our study suggests a common epigenetic pathogenic mechanism.

The main limitation of our study is the relatively small number of samples, stemming from the low incidence of sporadic NFPanNET and especially the hereditary types. The comparison between methylation status of the NFPanNET to NI samples may be affected by sample purity. Impurity of NI samples may lead to false positive methylation difference in genes with differential expression in the pancreatic islet vs. exocrine pancreas (the probable

contaminant). To control for such bias, we performed independent comparison of methylation levels excluding the islet cell samples, and found hypermethylation of sporadic and MEN1-related NFPanNET and hypomethylation of the VHL-related tumors, which supports the results of the comparison between NFPanNETs and NI samples.

In conclusion, our study supports the key role of altered DNA methylation in NFPanNET tumorigenesis, which was further supported by the associated gene expression changes in the differentially methylated genes. The methylome of NFPanNETs is distinct from NI and between VHL and sporadic and MEN1-related NFPanNETs. The involvement of common pathways in genes with altered methylation status suggest that epigenetic cancer therapy may be an effective treatment strategy for NFPanNETs that fail standard therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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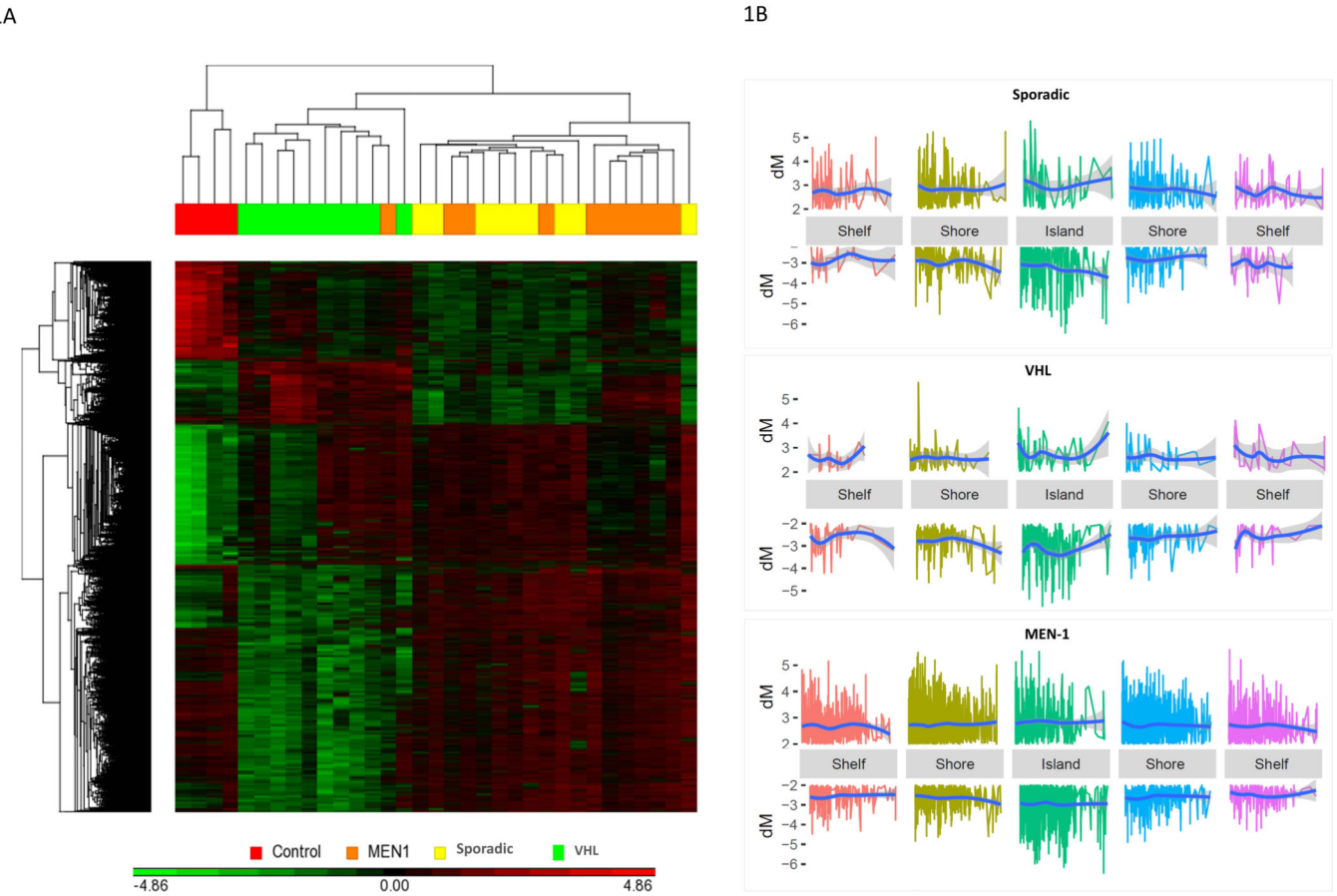
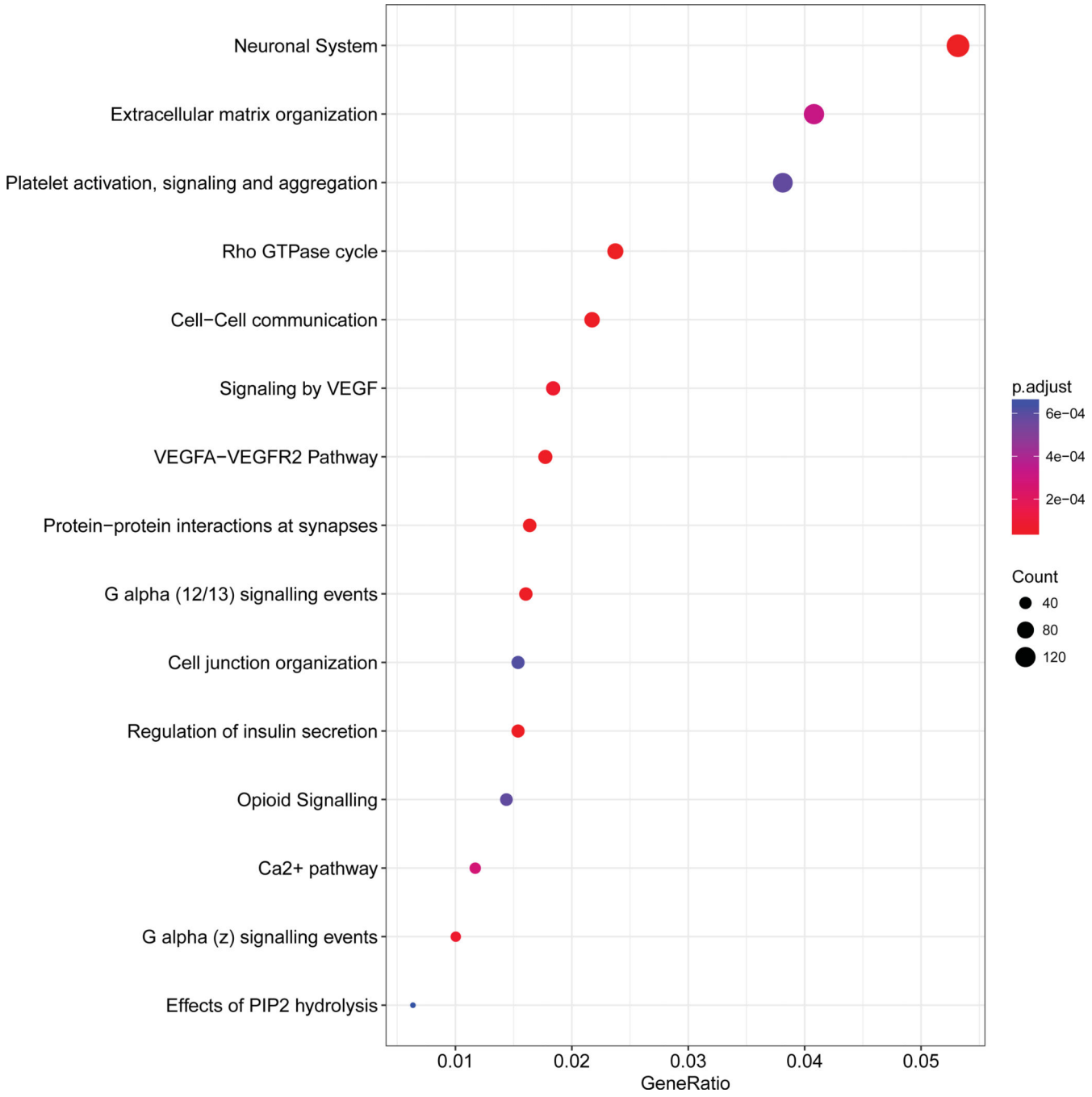


Figure 1. (A)

Heatmap of unsupervised cluster analysis based on methylation M values for the top 5000 most variable methylated probes. The sporadic and MEN1-related tumors clustered together and VHL-related NFPanNET separately. (B) DMPs by genomic regions of CpG sites. For each group upper/lower panels represent hyper/hypomethylation, respectively, the y-axis represents delta-M value (dM) vs. normal pancreatic islet-cells, the blue line (grey area) represents mean (standard deviation) of dM.

DMP, differentially methylated probes; NFPanNETs, non-functioning pancreatic neuroendocrine tumor; VHL, von Hippel-Lindau; MEN1, multiple endocrine neoplasia type 1; dM, delta-M value



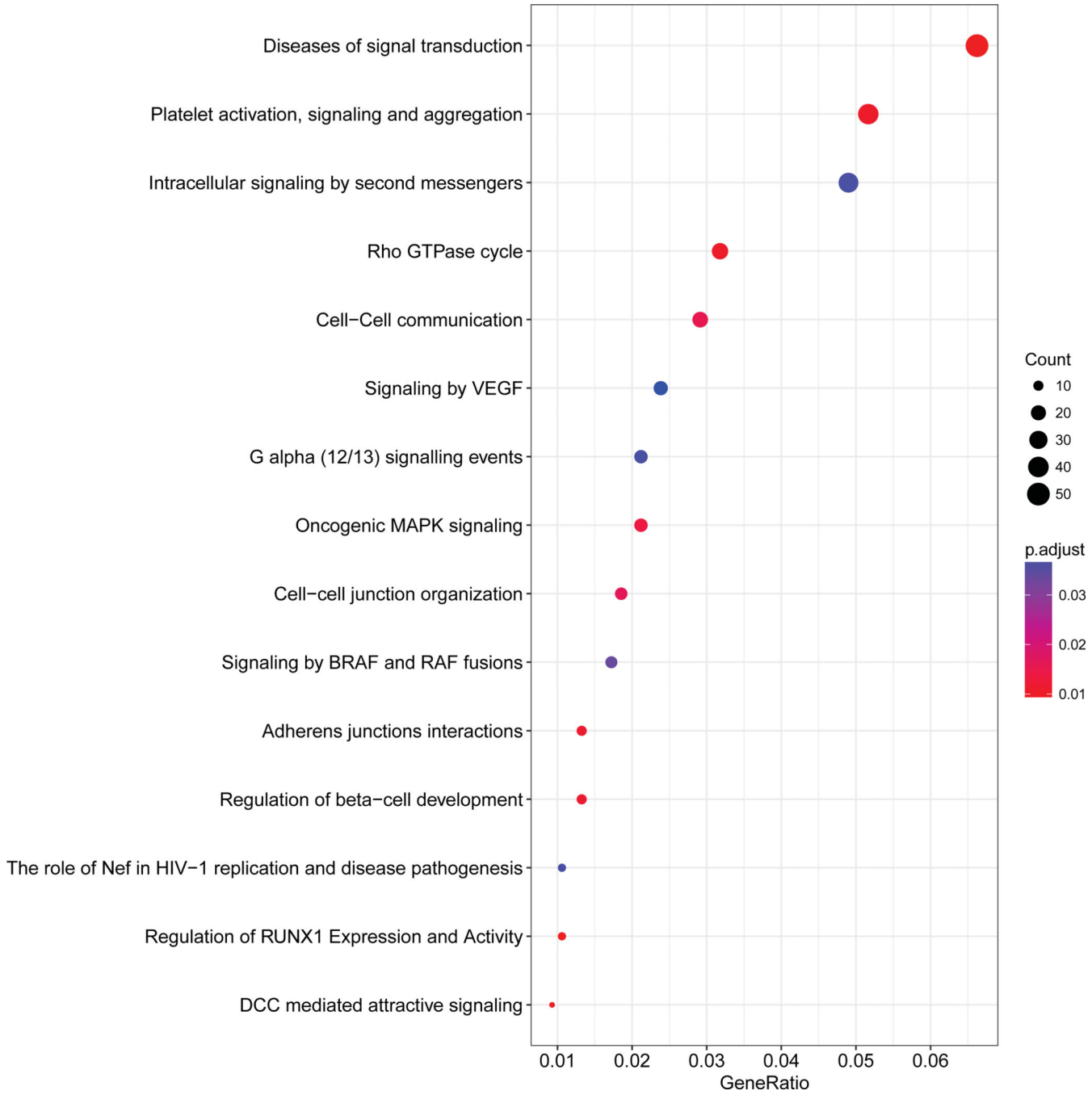
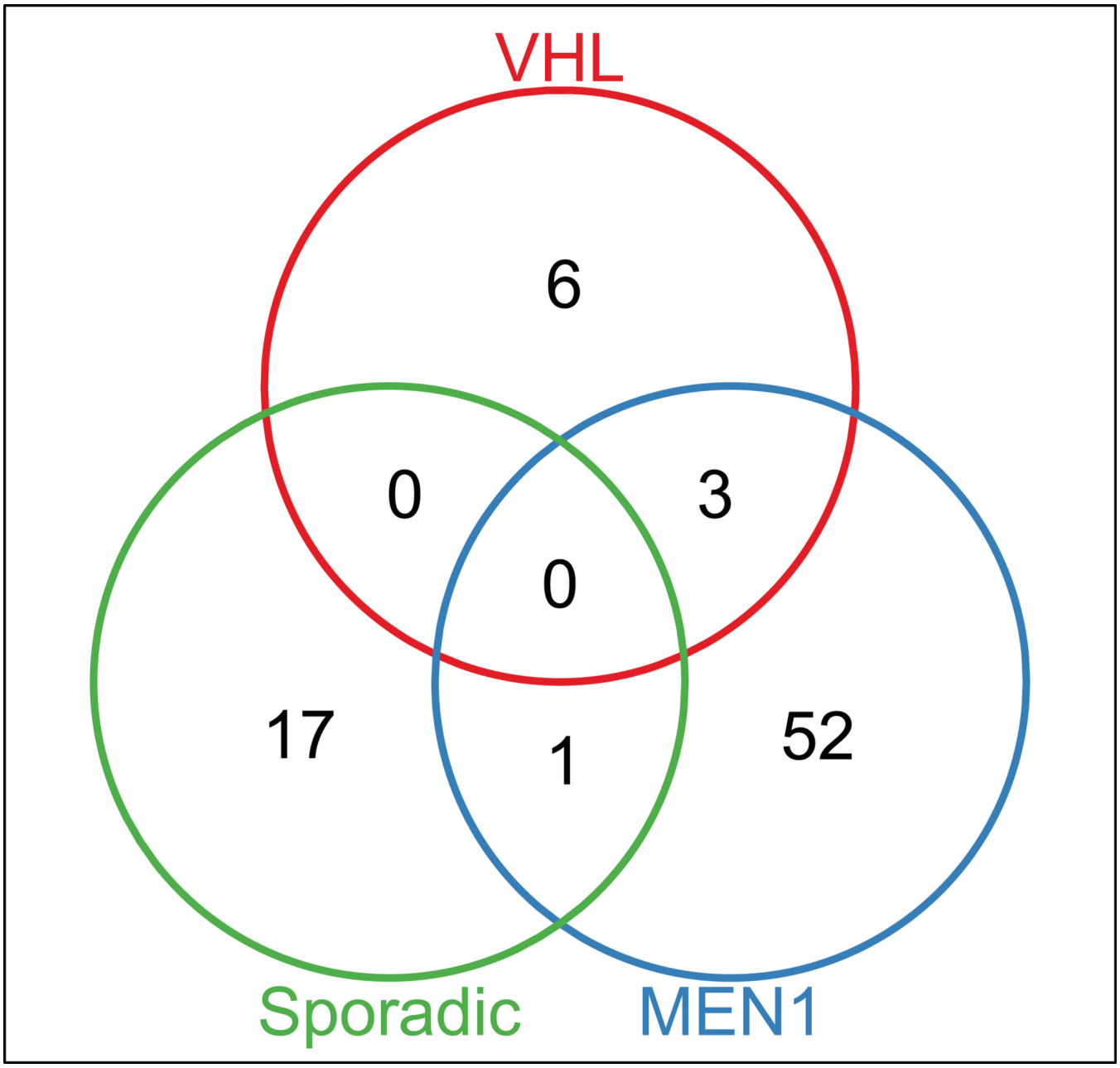
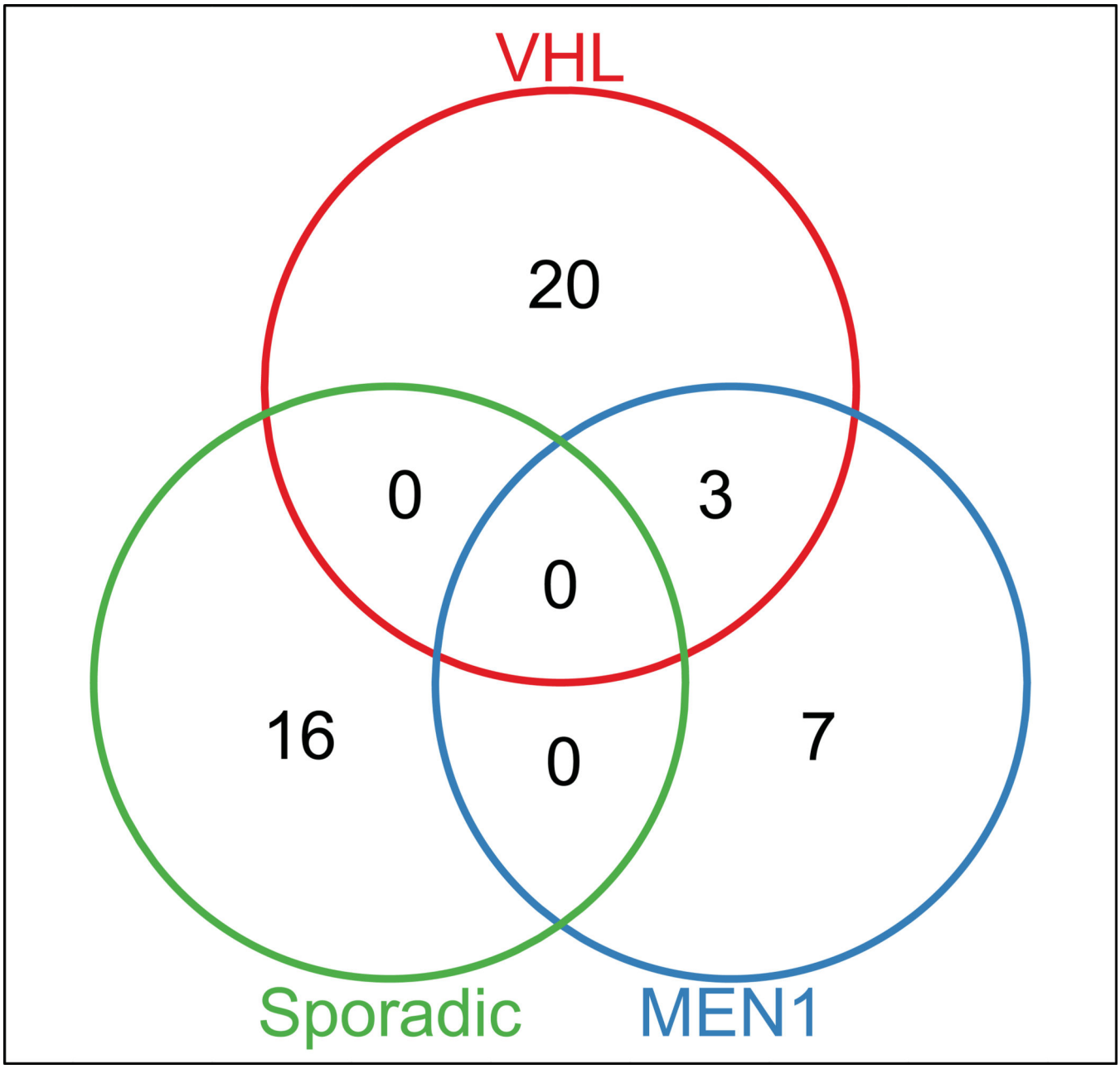


Figure 2.

Pathway analysis based on the differentially methylated probe analysis. For each group, the fifteen most enriched pathways are shown.

NFPanNETs, non-functioning pancreatic neuroendocrine tumor; VHL, von Hippel-Lindau; MEN-1, multiple endocrine neoplasia type 1





B

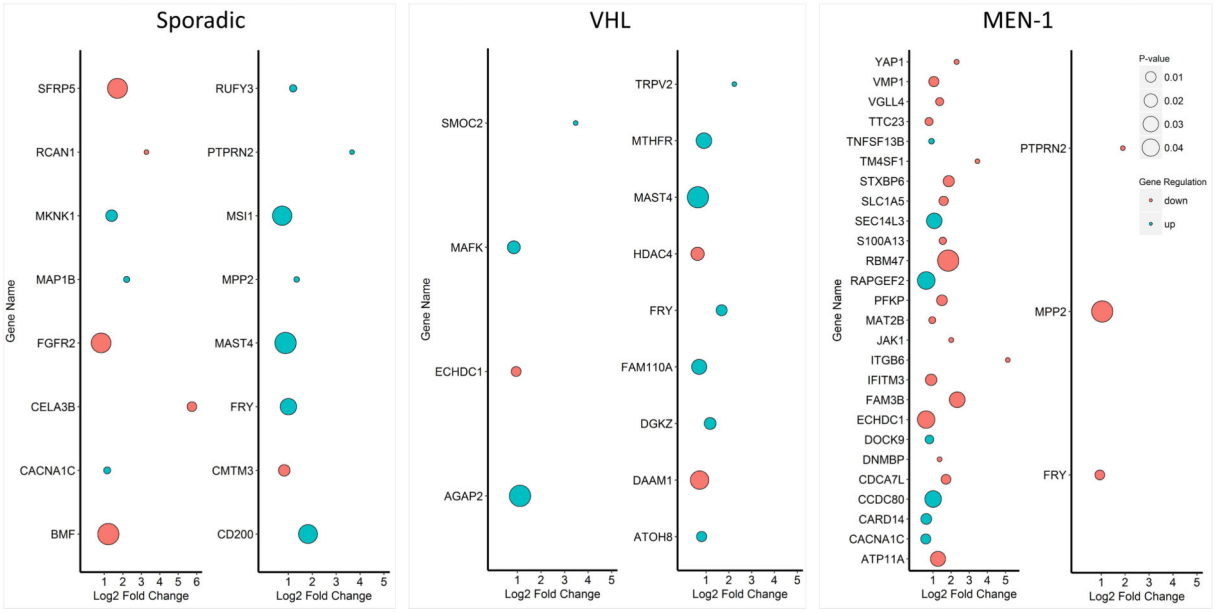
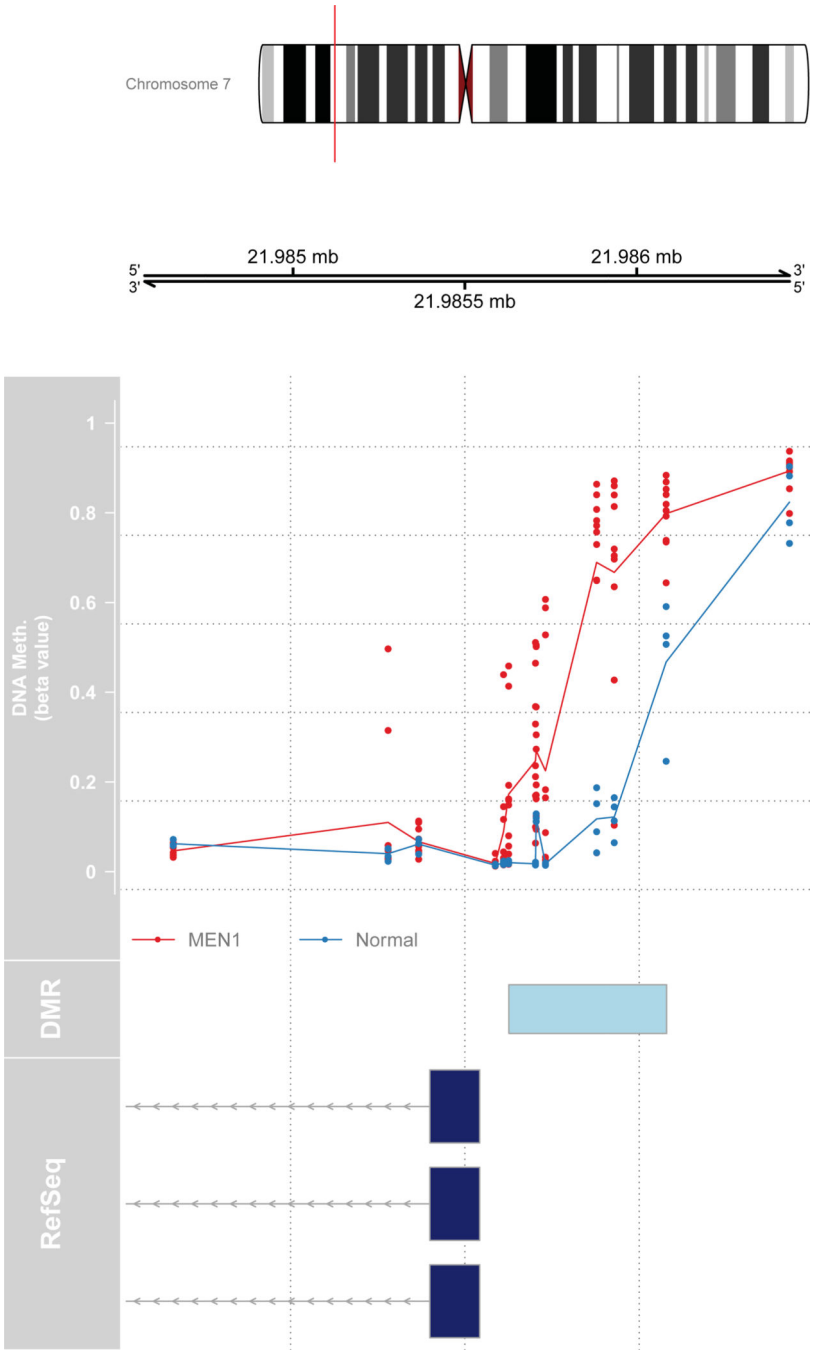
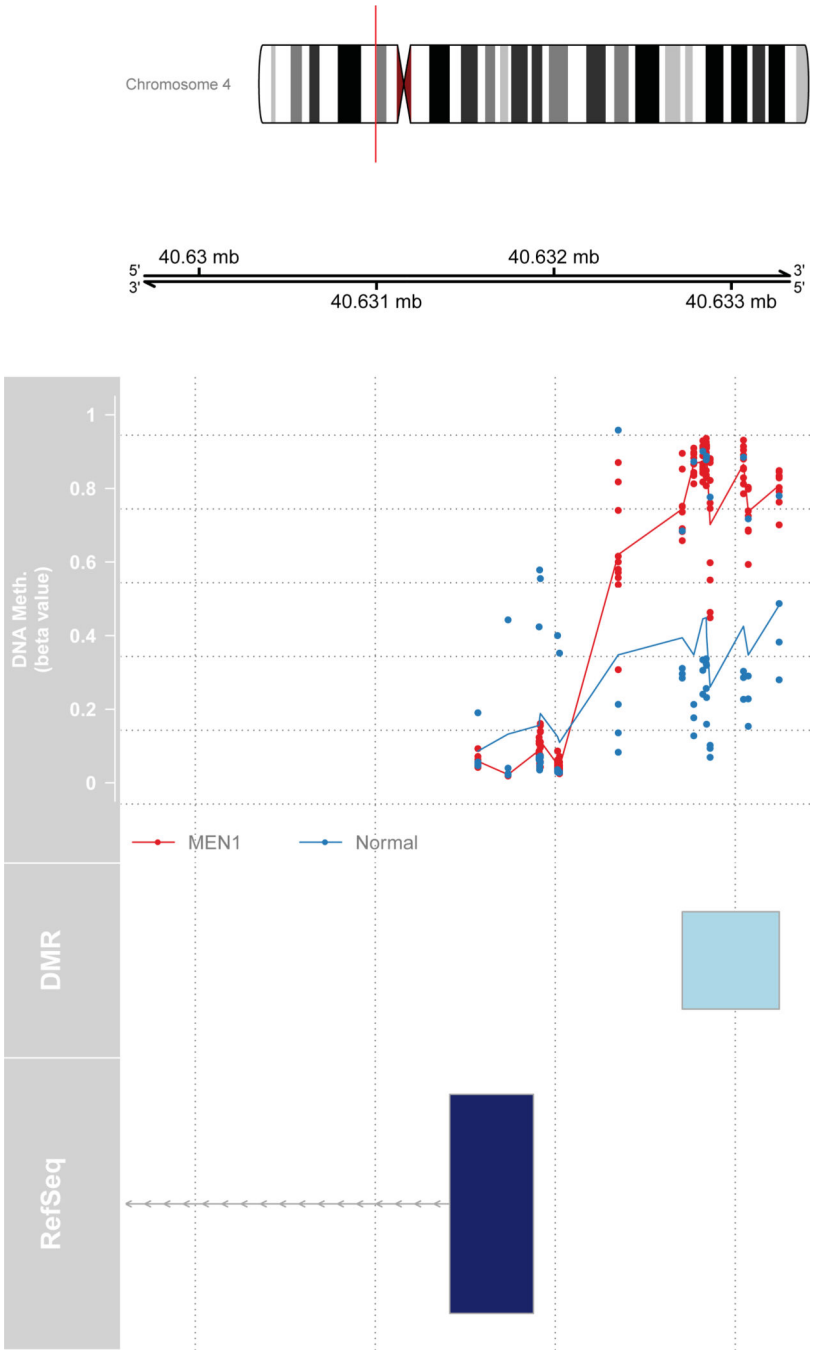


Figure 3.

Number of overlapping genes with hyper- and hypomethylation between sporadic, VHL and MEN1 NFPanNETs (**A**), and gene expression difference in NFPanNETs vs. normal islet cells, in the different groups (**B**), in significantly hyper and hypomethylated genes based in DMR analysis (left and right in each group, respectively). The filled color indicates up- (blue) or down-regulation (red) of gene expression.

NFPanNETs, non-functioning pancreatic neuroendocrine tumors; VHL, von Hippel-Lindau; MEN-1, multiple endocrine neoplasia type 1; DMR, differentially methylated regions





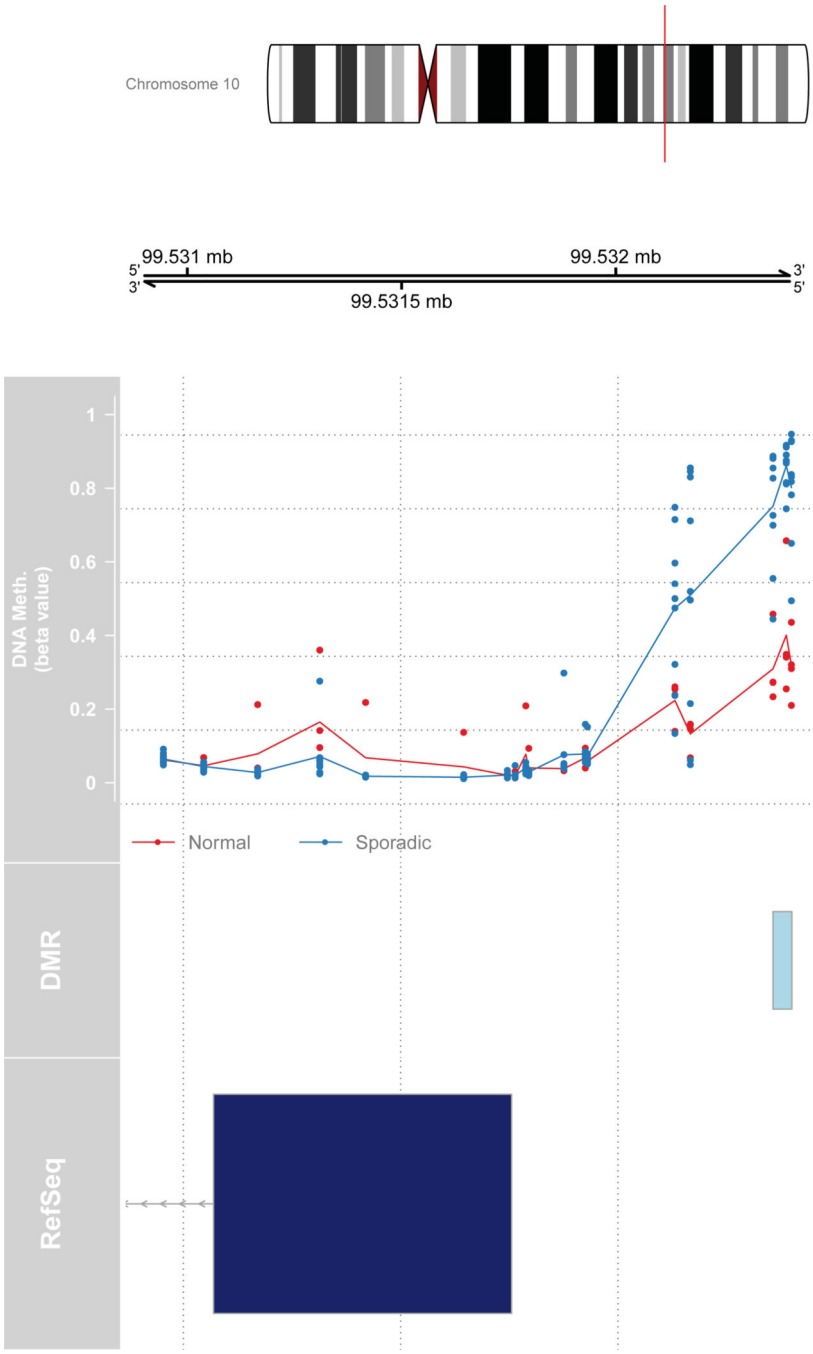


Figure 4.

Gene promoter hypermethylation in sporadic NFPanNET (*SFRP5*) and MEN-1 NFPanNET (*CDCA7L* and *RBM47*), compared with normal islet cells. The methylation status of the genes was inversely associated with differential gene expression.

NFPanNETs, non-functioning pancreatic neuroendocrine tumors; VHL, von Hippel-Lindau; MEN-1, multiple endocrine neoplasia type 1; DMR, differentially methylated regions

Table 1.

Clinical and histopathologic data of patients with NFPanNET in the study cohort

	Sporadic	VHL	MEN-1
Tumor WHO grade (Grade 1/Grade 2) n[%]	5/4 [56/44]	6/4 [60/40]	5/5 [50/50]
Metastatic disease (Yes/No/Unknown) n[%]			
Lymph nodes	0/9/0 [0/100/0]	1/6/3 [10/60/30]	2/6/2 [20/60/20]
Distant metastases	0/9/0 [0/100/0]	1/9/0 [10/90/0]	2/8/0 [20/80/0]
Tumor diameter (cm) median[range]	3.5 [3.0–7.0]	2.0 [0.9–5.0]	2.5 [0.7–3.2]
Tumor site in the pancreas (Head/Uncinate/Body/Tail) n[%]	6/0/1/2 [60/0/10/20]	5/1/1/3 [50/10/10/30]	3/1/1/5 [30/10/10/50]

NFPanNET, non-functioning pancreatic neuroendocrine tumor; VHL, von Hippel-Lindau; MEN-1, multiple endocrine neoplasia type 1; WHO, World Health Organization

Table 2

Gene enhancers with significantly different methylation status in non-functioning pancreatic neuroendocrine tumors.

Group	Methylation status	Coordinate	dbSNP ID	RegulomeDB score	Gene regulated
VHL	Hypo	chr7 157361733	rs221294	1b	*PTPRN2*
VHL	Hypo	chr17 16318931	rs3813769	1f	*TRPV2*
Sporadic	Hypo	chr7 157361733	rs221294	1b	*PTPRN2*
MEN-1	Hyper	chr19 47288149	rs8105903	1a	*SLC1A5*
MEN-1	Hyper	chr1 18807136	rs2992757	1d	*PQLC2*
MEN-1	Hypo	chr7 157361733	rs221294	1b	*PTPRN2*

VHL, von Hippel-Lindau; MEN-1 multiple endocrine neoplasia type 1 syndrome