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Original Article

DNMT3B modulates the expression of cancer-related genes and downregulates the expression of the gene VAV3 via methylation

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Abstract: Altered promoter DNA methylation is one of the most important epigenetic abnormalities in human cancer. DNMT3B, *de novo* methyltransferase, is clearly related to abnormal methylation of tumour suppressor genes, DNA repair genes and its overexpression contributes to oncogenic processes and tumorigenesis *in vivo*. The purpose of this study was to assess the effect of the overexpression of DNMT3B in HaCaT cells on global gene expression and on the methylation of selected genes to the identification of genes that can be target of DNMT3B. We found that the overexpression of DNMT3B in HaCaT cells, modulate the expression of genes related to cancer, downregulated the expression of 151 genes with CpG islands and downregulated the expression of the VAV3 gene via methylation of its promoter. These results highlight the importance of DNMT3B in gene expression and human cancer.

Keywords: Methylation, *de novo* methyltransferase, overexpression of DNMT3B, cancer, cancer-related genes, VAV3, CpG island

Introduction

Epigenetic and genetic alterations are common in the genesis and progression of various types human cancer. The abnormal expression of genes related to cell cycle, DNA repair, cellular metabolism and tumor suppressor are frequent defects that contribute to development of cancer [1]. Abnormal DNA methylation is one of the most important epigenetic factors directly involved in tumourigenesis, because methylation can induce repression of tumor suppressor genes or activation of oncogenes [2].

In human cancer the patterns of DNA methylation are altered: the overall level of DNA methylation is lower in normal cells than in cancer cells and the methylation of CpG islands of tumor suppressor and DNA repair is higher in cancer than normal cells [3]. DNA methylation at the 5' cytosine of CpG sites is catalyzed by DNA methyltranferases (DNMTs). The DNMT family includes three enzymes, DNMT1 responsible for maintaining pre-existing methylation patterns after DNA replication and DNMT3A and DNMT3B, *de novo* methyltransferases that are required to establish methylation during development and imprinting [4, 5]. Genetic abnormalities and aberrant overexpression of DNMTs contribute to DNA hypermethylation in cancer [6, 7]. Inhibition of these enzymes in cancer can decrease DNA methylation, reactivate silence genes and diminish tumorigenicity [8]. Furthermore, it has been showed that DNMT3B is overexpressed in cell lines of cancer and in several types of primary tumors [9-14]. In several works of cancer, it has has been reported that there is a positive correlation between DNMT3B expression and promoter DNA methylation [11, 13, 15, 16]. Interestingly, DNMT3B contributes to oncogenic processes and tumorigenesis *in vivo* by gene-specific *de novo* methylation and transcriptional silencing [17]. Overexpression of DNMT3B protein significantly contributes to elevated methyltransferase activity and hypermethylation in

breast cancer cells [13]. Although, the important role of DNMT3B in cancer development is clear, at present only a few genes have been identified as targets for transcriptional regulation by this enzyme [18-21].

Therefore, the purpose of this study was to assess the effect of the overexpression of DNMT3B in HaCaT cells on global gene expression and on the methylation of selected genes to the identification of genes that can be target of DNMT3B. We found that the overexpression of DNMT3B in HaCaT cells downregulated the expression of VAV3, SORBS2, and GPR137 genes by microarray and RT-qPCR and a clear increase in DNA methylation was detected in VAV3 promoter.

Materials and methods

Cell culture and cervical samples

The HaCaT (human skin keratinocyte), C-33A (cervical cancer), HeLa (cervical cancer), SiHa (cervical cancer), A549 (lung adenocarcinoma) and MCF-7 (breast adenocarcinoma) cells lines were obtained from American Type Culture Collection (ATCC, USA), cultured in DMEM and F-12 1:1, medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. The cells were grown at 37°C in 5% CO_2. The samples were collected at the Cancer Institute of the State of Guerrero located in southern Mexico. The population consisted of 25 healthy women and 25 women with cervical cancer. The diagnosis of normal cervix was done by cytomorphological examination through conventional Papanicolaou test and cervical cancer by histological diagnosis, according to the classification system of the International Federation of Gynecology and Obstetrics (FIGO). All samples were obtained after the patients gave their informed consent and the Bioethics and Research Committee of the Cancer Institute of the State of Guerrero, Mexico, approved the study, which followed the ethical guidelines of the 2008 Helsinki Declaration.

Transient transfection

Complementary DNA encoding DNMT3B was cloned into pcDNA3.1(+) plasmid (Invitrogent, Carlsbad, CA USA) to generate the pcDNA-DNMT3B expression plasmid that was confirmed by sequencing. The HaCaT cells (25 x 10^3 cells, 6-well plates) were transfected with Lipofectamine 2000 Reagent (Invitrogent) according to the manufacturer's protocol. The cells were transfected with 3.5 µg of pcDNA-DNMT3B plasmid or empty vector pcDNA3.1(+) and after 48 h the cells were harvested for RNA and DNA extraction.

RNA and DNA extraction

Total RNA was isolated and purified from the cell lines and cervical tissue with Direct-zol RNA MiniPrep (ZYMO Research, Irvine, USA) according to the manufacturer's instructions including DNase I treatment. RNA integrity was determined by electrophoresis in a 1% agarose gel. Genomic DNA was extracted from the cells using a standard phenol chloroform method [22]. The concentration of RNA and DNA was evaluated by spectrophotometry using NanoDrop 2000c (Thermo Scientific, Wilmington, DE USA).

Microarray analysis

H35K array was performed in Microarray Unit of Cellular Physiology Institute, UNAM, Mexico City. H35K contains 70-mer oligonucleotide probes representing 35764 human transcripts. Total RNA was extracted of HaCaT cells transfected with pcDNA-DNMT3B and of HaCaT cells transfected with pcDNA3.1(+) (empty vector). Equimolar concentrations of total RNA from of 3 independent experiments were mixed. Ten µg of RNA were used for cDNA synthesis and equal quantities of Cy3-labeled cDNA from control cells and Cy5-labeled cDNA from experimental cells were hibridized to the H35K array. Each hybridization was carried out in duplicate. Array signal intensities were analyzed with ScanArray 4000 from Packard BioChips. Microarray data analysis, background correction, normalization and selection of differentially expressed genes were performed with GenArise software (http://www.ifc.unam.mx/genarise/). Differentially expressed genes were selected according to the Z-score value [23]. Differential expressed genes were considered upregulated when Z-score > 1.5 standard deviation or downregulated when Z-score < 1.5 standard deviation.

Bioinformatics analysis

Gene ontology (GO) analysis of the differentially expressed genes was performed with PANTHER (http://www.pantherdb.org/) and according to

Table 1. Primer sequences used in this study

Gene	Sequence	Tm °C
RT-qPCR		
MSH2	F5'-TTCATGGCTGAAATGTTGGA	59
	R5'-ATGCTAACCCAAATCCATCG	
NSD1	F5'-TGAAGGCAGACATCAATTCG	55
	R5'-CCAACTTGATTGAACCAGGAA	
SORBS2	F5'-AAGCACAGCCTGCAAGACCA	60
	R5'-TGGGGTATTGGAGGGTCAGG	
ARHGAP29	F5'-TTAGAGGATGTTGTACGCC	58
	R5'-TTCGATGAAAGTCTCCTGG	
VAV3	F5'-ACAAGGAGCCAGAACATTCAG	58
	R5'-TTGCACAGAAGTCATACCGAG	
GPR137	F5'-TCAGCTATCAGACGGTGTTC	52
	R5'-AGCAGTAGAGAAGCCAGAAG	
C1ORF201	F5'-CTTGTGAAGCAGTCGCCAAATACAT	58
	F5'-CACGATCTCATACTGACCAGGACCT	
THSD1	F5'-GGAGGCCAACACCAATCAGA	59
	R5'-CAGTAGTCACCAGCCTCCTT	
ST6GALNAC2	F5'-GGGTCGTTCTTCTGGCTGCT	59
	R5'-TGATGTGGTGTCCCTGGCTC	
MSX1	F5'-CCAGAAGATGCGCTCGTCAA	59
	R5'-TCGTCTTGTGTTTGCGGAGG	
GAPDH	F5'-CCGGGAAACTGTGGCGTGATGG	60
	R5'-AGGTGGAGGAGTGGGTGTCGCTGTT	
MSP		
VAV3-R1 M	F5'-GTTTTGGGGGATTTTATCGTATTAT	58
	R5'-GACCCGCCACTAAACATACCCAAC	
VAV3-R1 U	F5'-TGGGGGATTTTATTGTATTATAGTA	55
	R5'-AACCCACCACTAAACATACCCAACA	
VAV3-R2 M	F5'GGCGTTGGAGTCGGAAGTTTGTG	60
	R5'-CACTACTTCCACGACTCCATACC	
VAV3-R2 U	F5'-GGTGTTGGAGTTGGAAGTTTGTGT	59
	R5'-CACACACTACTTCCACAACTCCATACC	
SORBS2-R1 M	F5'-ATAATAAAAGAATAAATTTAGGTCGGG	58
	R5'-CTATCGCCCAAACTAAAATACAAT	
SORBS2-R1 U	F5'-TATAATAAAAGAATAAATTTAGGTTGGG	54
	R5'-AAAATAAAATCTCACTCTATCAC	
SORBS2-R2 M	F5'-GGGAATTATGTGTTAATTTAATTCG	52
	R5'-AAATCATAAATACTAAACGCTCC	
SORBS2-R2 U	F5'-GGAATTATGTGTTAATTTAATTTGATG	56
	R5'-ATAAAATCATAAATACTAAACACTCC	
BSP		
VAV3-R1	F5'-AGGGGGTTTTGGGGGATTTTAT	56
	R5'-CCACTAAACATACCCAACA	
VAV3-R2	F5'-GGCGTTGGAGTCGGAAGTTTGTG	60
	R5'-CACTACTTCCACGACTCCATACC	
SORBS2-R1	F5'-AGTTATAAAATTTTGATTGGTTGA	58
	F5'-AACCTACAAACTTACTCTAAATCCTAT	

the program an enrichment score of $P < 0.05$ was considered as significant. For promoter prediction we considered 3000 pb (-2000 pb to +1000 pb) relative to ATG using the ExPASy Bioinformatics Resource Portal (http://www.expasy.org/genomics). For CpG island prediction the criteria was regions > 200 bp with a GC content ≥ 50% with an observed CpG/expected CpG > 0.6 [24]. CpG islands prediction was done using the Methprimer Program (http://www.urogene.org/methprimer/). The prediction of transcription factors that can bind to VAV3 promoter was done with CONSITE database (http://consite.genereg.net/).

RT-qPCR

One hundred ng of total RNA were used in each RT-qPCR assay. Reverse transcription and quantitative PCR were performed with KAPA SYBR FAST One-Step qRT-PCR kit (Kapa Biosystems, Boston, Massachusetts, USA), according to the manufacturer's protocol. In all cases, the conditions of reverse transcription and amplifications were: 30 s at 37°C, 42°C for 5 min and 95°C for 5 min; 40 cycles of amplification: 5 s at 95°C, 30 s at 60°C and 30 s at 72°C; melt curve: 15 s at 95°C, 1 min at 60°C and 15 s 95°C. The reactions were done in Real Time ABI-PRISM 7500 SDS (Applied Biosystems, Foster City, CA). Data were normalized using GAPDH as an internal control and relative expression differences were calculated using the $2^{-\Delta\Delta Ct}$ method. Primers sequences are shown in **Table 1**.

Methylation-specific PCR (MSP) and bisulfite sequencing (BSP)

For MSP, 1 μg of DNA was treated with sodium bisulfite using the EpiTect Bisulfite kit (QUIAGEN, Hilden, Germany) according to the manufacturer's instructions. MSP

SORBS2-R2	F5'-GGAATGATGTTTATAGGGAATTATGTG F5'-CCCTAAAAATAAAATCATAAATACTAAA	59
GPR137-R1	F5'-GGGGGTATTGGAGATAAGGAAAGG F5'-CTCCTCTCTCCTATACCCAAATC	59
GPR137-R2	F5'-TTTTTTTTTTTTGAGGTTGGAG F5'-CAAACCCCTCACTCAAAAACA	59

primer sequences are shown in **Table 1**. MSP was performed in a total of 10 μL, containing 1 μL of bisulfite-treated DNA, 250 nM of each primers and AmpliTaq Gold360 Master Mix (Applied Biosystems) and under the following amplification conditions: denaturation 95°C for 10 min, 40 cycles of amplification: 30 s at 95°C, 30 s at 60°C and 30 s at 72°C, and a final extension of 72°C for 10 min. Bisulfite sequencing was done for VAV3, SORBS2, and GPR137 genes. The promoters of these genes were divided into two regions to facilitate the methylation analysis. One hundred ng of bisulfite-treated DNA was used as a template, and PCR was performed using specific primers (**Table 1**). The reactions were done in Eppendorf Mastercycler EP Gradient 96 Thermal cycler (Applied Biosystems). The PCR products were gel purified and cloned into the pJET1.2/blunt vector (Thermo Scientific). Five independent clones were subjected to automated sequencing (ABI Prism 310 Genetic Analyzer (Applied Biosystems).

Statistical analysis

The data are shown as mean ± standard deviation. The *P* value was determined using Student's *t*-test. *P* values below 0.05 were considered statistically significant.

Results

DNMT3B has an important role in aberrant DNA methylation to repress transcription. To identify downregulated genes by DNMT3B, we overexpressed DNMT3B in the HaCaT cell line, and H35K microarray that interrogated 35764 genes was used to identify changes in gene expression. We found 1085 downregulated genes, 1741 upregulated genes and 32938 unchanged genes (**Figure 1A**). To gain insights into the biological processes where 1085 downregulated genes are implicated, we carried out a gene ontology (GO) analysis using Protein Analysis Through Evolutionary Relationships (PANTHER). This analysis revealed that an important part of the 1085 downregulated genes are involved in the immune system, development processes, cell communication, cellular processes and metabolic processes (**Figure 1B**). The GO analysis for the 1741 upregulated genes is shown in Supplementary Figure 1.

The 1085 downregulated genes were classified according to Z-score value (**Figure 2A**). We narrowed down this group of genes by the selection of gene subsets with Z-scores of -2 to -6.8 (252 genes). Hypermethylation of CpG islands found within promoters is clearly related to transcriptional repression. Therefore, to relate the 252 downregulated genes with the methylation of its promoter by overexpression DNMT3B, we used MethPrimer to prediction of CpG islands for 252 genes. We found 151 genes with CpG islands, 73 genes without CpG islands and 28 genes with absent data (**Figure 2B**). To know the biological processes where 151 genes with CpG islands are involved, we carried out GO analysis. We found that some of these genes are implicated in molecular and cellular processes altered in cancer such as adhesion, apoptosis, response to stimulus, development, biological regulation and metabolic processes (**Figure 2C**). Among the 151 genes with CpG islands, we find genes with previous reports of abnormal methylation in several types human tumors, many genes putative or tumor suppressor and genes related with cancer. The complete list of 151 genes with CpG islands is shown in Supplementary Table 1.

To validate the results of the microarray, we analyzed the level of expression of 10 genes by RT-qPCR. These 10 genes were selected for further validation because 1) they were downregulated by overexpression of DNMT3B, 2) they have CpG islands and 3) they are involved in regulating important molecular and cellular functions which are disrupted in cancer. The function of 10 genes is shown in Supplementary Table 2. The level of expression of 7 genes was consistent with data from microarray analysis and inconsistent in three genes (**Figure 3**). The analysis by RT-qPCR showed that expression levels of SORBS2, VAV3 and GPR137 mRNAs were significantly downregulated by the overexpression of DNMT3B.

To clarify whether downregulation of VAV3, SORBS2, and GPR137 is mediated by DNA hypermethylation in overexpression of DNMT3B

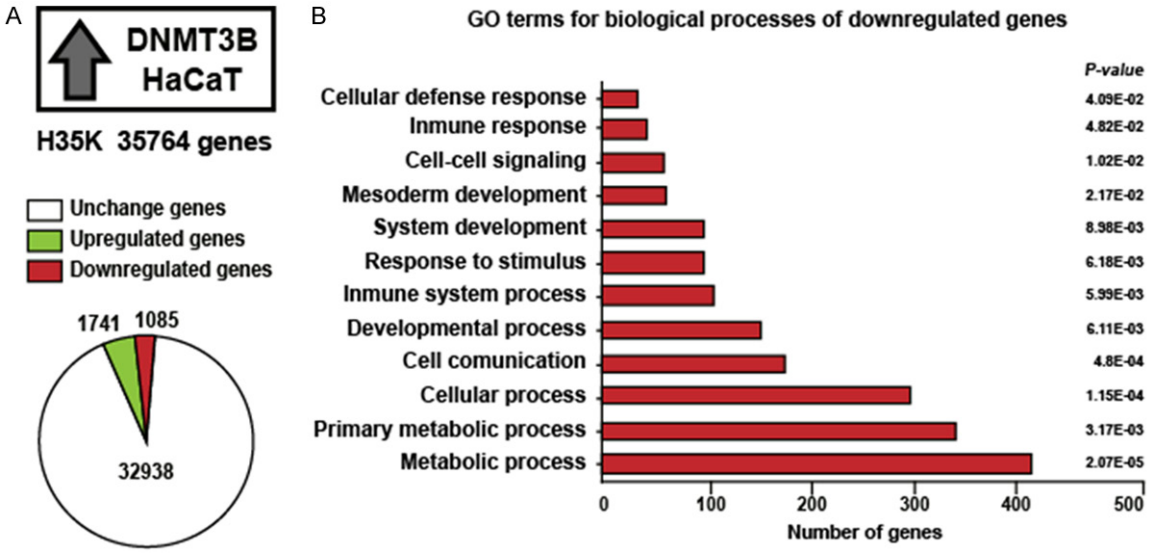

Figure 1. Gene ontology analysis of downregulated genes by overexpression of DNMT3B in HaCaT cells. A: We used H35K array of 35764 genes, the graph shows the number of genes that change their expression by overexpression of DNMT3B. B: Gene ontology (GO) analysis for downregulated genes by overexpression of DNMT3B.

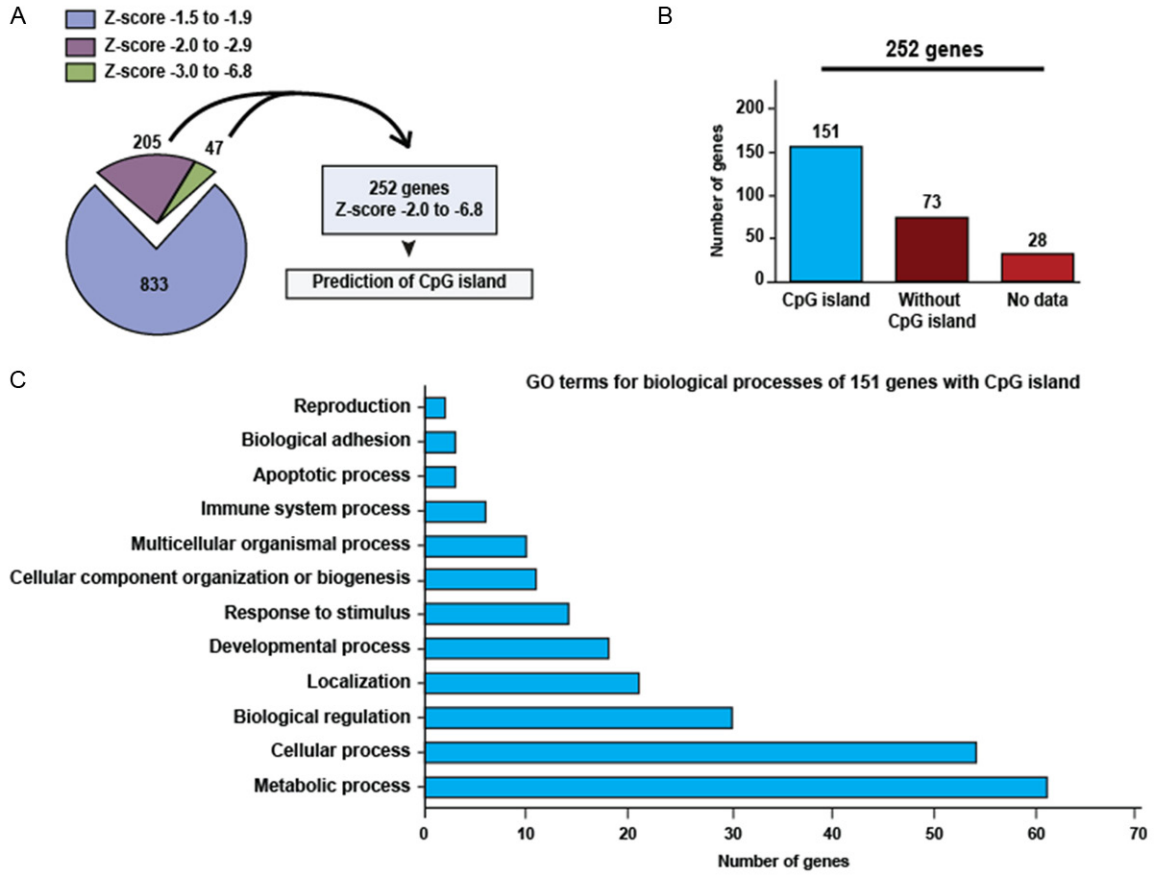

Figure 2. Prediction of CpG island in downregulated genes by overexpression of DNMT3B in HaCaT cells. A: Classification of downregulated genes according to Z-score value, the graph shows the number of genes for each Z-score range. B: Number of genes with and without CpG island. C: Gene ontology (GO) analysis for 151 genes with CpG island.

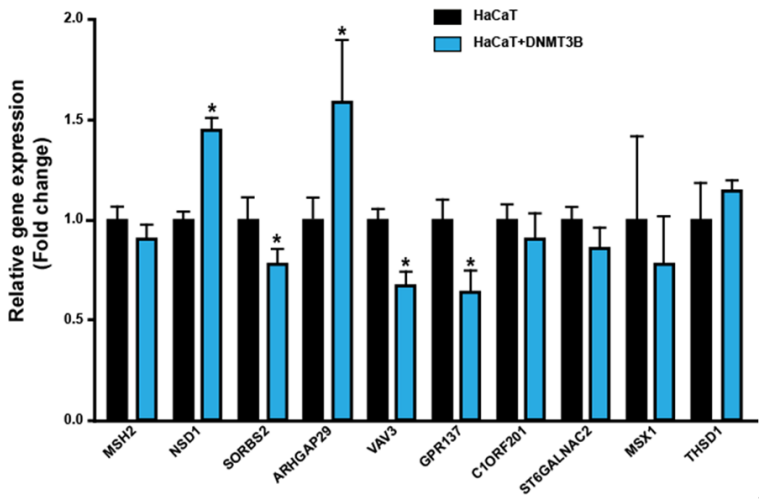

Figure 3. Validation of microarray data by RT-qPCR. mRNA quantification of 10 genes in HaCaT cells with overexpression of DNMT3B and control HaCaT cells. The bars represent the mean ± standard deviation from at least three independent experiments. *$P < 0.05$

HaCaT cells, we analyzed the methylation status of its promoters by using methylation-specific PCR (MSP) and bisulfite conversion and sequencing. For the VAV3 gene, its CpG island spanning from -599 pb to +20 pb of the transcription start site, within of this region we found 95 CpGs sites (**Figure 4A**). No obvious methylation changes were observed between HaCaT cells and HaCaT cells with overexpression of DNMT3B by MSP analysis (**Figure 4B**). To make a more detailed analysis of methylation status, we analyzed the methylation in the 95 CpGs sites of the VAV3 promoter. We found two small, more densely methylated regions (15, 16, 17, 18, 19 and 21 CpG sites of region 1 and 52, 53, 54, 55, 56, 57, 58 and 59 CpG sites of region 2) of the VAV3 promoter in HaCaT cells with overexpression of DNMT3B in comparison with HaCaT cells (**Figure 4C**). These results suggest that the overexpression of DNMT3B in HaCaT cells probably has a role in the methylation of the VAV3 promoter. The MSP and bisulfite conversion and sequencing analysis was done for SORBS2, and GPR137 genes but no methylation changes were observed between HaCaT cells with overexpression of DNMT3B and HaCaT cells (Supplementary Figures 2 and 3).

Finally, to correlate our results with what occurs in human cancer, we analyzed the expression of DNMT3B in cervical cancer samples and normal cervical tissue. As well as DNMT3B, VAV3, SORBS2 and GPR137 expression in cervical, lung and breast cancer cell lines. RT-qPCR analysis showed that mRNA level of DNMT3B in cervical cancer samples was significantly higher that in normal tissue (**Figure 5A**). In general, in the analyzed cell lines, we found overexpression of DNMT3B and low levels VAV3, SORBS2 and GPR137 (**Figure 5B**). These results suggest that overexpression of DNMT3B can be a common event in human cancer and expression of VAV3, SORBS2 and GPR137 could be regulated by DNMT3B.

Discussion

DNMT3B overexpression and abnormal methylation of tumour suppressor and DNA repair genes are common alterations in several types of human cancer [6, 25]. There is evidence indicating the involvement of DNMT3B in the initiation and progression of cancer [20, 26]. In addition DNMT3B is clearly related to the abnormal methylation in cancer [21, 27]. Although only 5 genes have been identified as targets for transcriptional repression by DNMT3B [18-21].

In this work the overexpression of DNMT3B in HaCaT cells downregulated 151 genes with CpG islands. This result suggests that the downregulated genes could be result from the methylation of its promoter by DNMT3B overexpression. In this sense, it has been reported that DNMT3B preferably to methylate CpG-dense promoter regions and is excluded from active promoters [28]. Also, downregulation or repression by methylation requires promoters with high methylated-cytocines [29-31]. In initiation and progression of cancer, DNMT3B has directly or indirectly been associated with abnormal expression and methylation [8, 26, 27]. An similar scenario it could be also seen in our study in which of the downregulated 151 genes by DNMT3B were found 22 genes with previous reported of abnormal methylation in several types of human cancer, 9 reported as putative or tumor suppressors genes and 61 genes related to many aspects of human cancer.

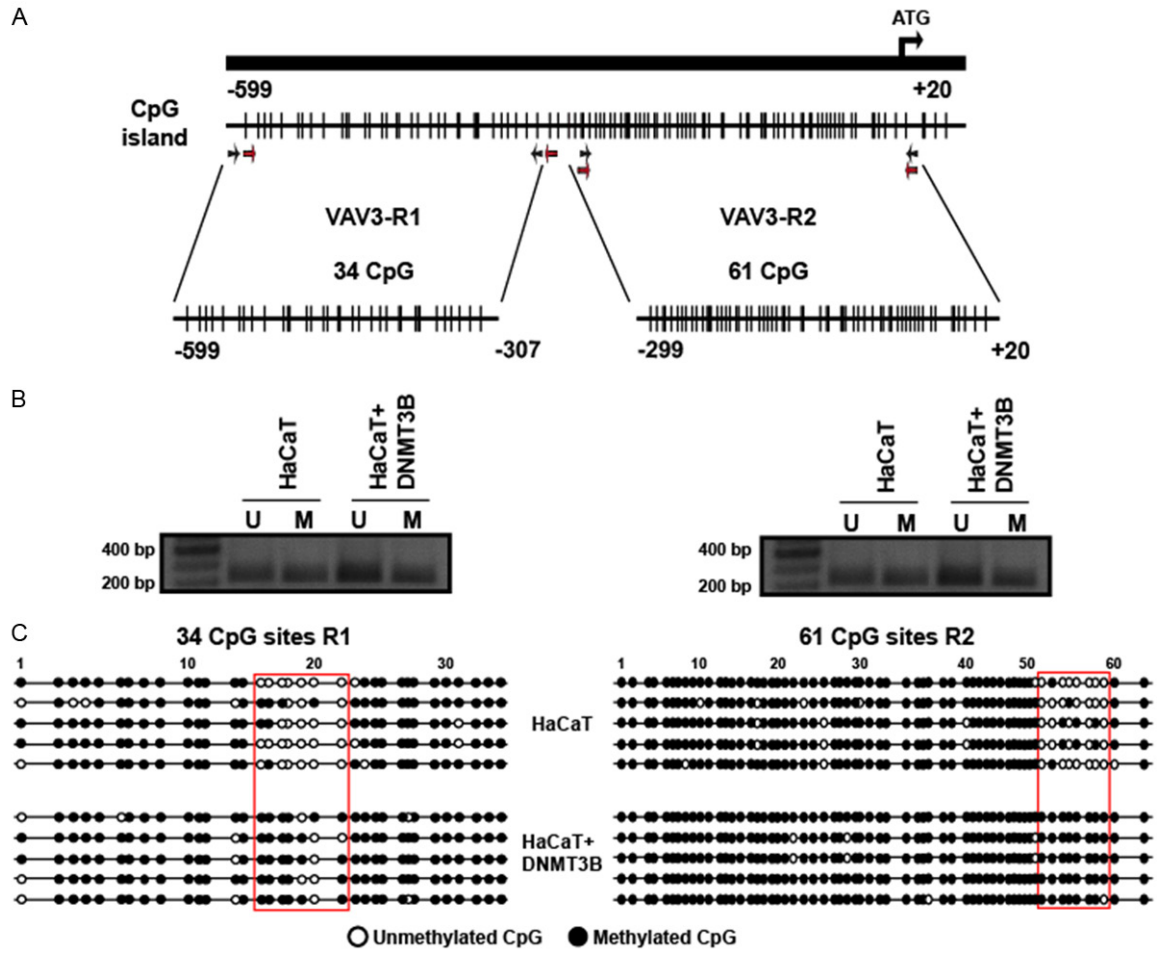

Figure 4. Methylation analysis of VAV3 promoter in HaCaT cells. A: Schematic representation of the CpG island and CpG sites in the VAV3 promoter. For methylation analysis the VAV3 promoter was divided into 2 regions: R1 -599 to -307 with 34 CpGs and R2 -299 to +20 with 61 CpGs, the positions are relative to the transcription start site. The primers for MSP and BSP are indicated by black and red arrows, respectively. Each CpG site is represented by a vertical bar. B: The methylation status of the VAV3 promoter (R1 and R2) was determined by MSP in HaCaT cells with overexpression of DNMT3B and control HaCaT cells. U showed unmethylation-specific primer amplification, M showed methylation-specific primer amplification. C: BSP analysis of the VAV3 promoter (R1 and R2) in HaCaT cells with overexpression of DNMT3B and control HaCaT cells. Black circles represent methylated CpG site and white circles represent unmethylated CpG site. The red box shows the two regions more densely methylated by overexpression of DNMT3B.

The overexpression of DNMT3B in HaCaT cells, downregulated the expression of VAV3, SORBS2, and GPR137 genes by RT-qPCR, but a clear increase in DNA methylation was only detected in the VAV3 promoter. Therefore it is possible that the VAV3 gene is regulated by DNMT3B via methylation of its promoter. VAV3 is a guanine nucleotide exchange factor involved in the regulation of Rho GTPases and in several cellular processes, including regulation of cytoskeleton organization, cell transformation and oncogenesis [32-34]. In addition, abnormal methylation of the VAV3 promoter has been reported in breast cancer cell lines and in gastric cancer the methylation of its promoter is considered as a marker to estimate the fraction of cancer cells in primary gastric cancer [35, 36]. On the other hand, we detected methylation of the VAV3 promoter in HaCaT cells without overexpression of DNMT3B. Although this result is unexpected, previously methylation of the VAV3 promoter in normal cells of the gastric mucosa has been reported [36]. By *in silico* analysis with CONSITE we detected that the transcription factors: Sp1, AP2 alpha, MZF, E2F, Hen-1 and Thing1-E47

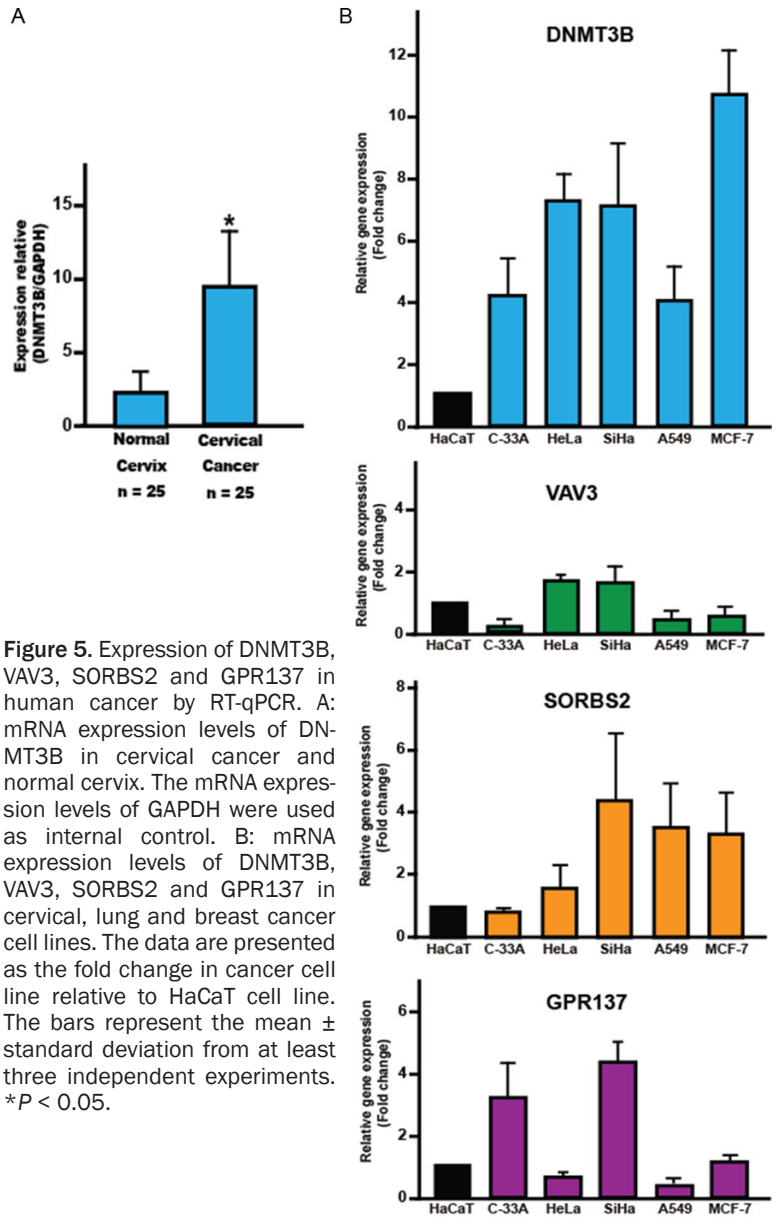

Figure 5. Expression of DNMT3B, VAV3, SORBS2 and GPR137 in human cancer by RT-qPCR. A: mRNA expression levels of DNMT3B in cervical cancer and normal cervix. The mRNA expression levels of GAPDH were used as internal control. B: mRNA expression levels of DNMT3B, VAV3, SORBS2 and GPR137 in cervical, lung and breast cancer cell lines. The data are presented as the fold change in cancer cell line relative to HaCaT cell line. The bars represent the mean ± standard deviation from at least three independent experiments. *$P < 0.05$.

can bind to localized sites in the more densely methylated regions of the VAV3 promoter. It is well known that the methylation of CpG in the Sp1 binding site generally interferes with its binding and can affect the transcription [37, 38]. The E2F transcription factor, does not bind DNA when their site recognition is methylated [39]. To some promoters AP2 alpha can act as a suppressor for Sp1 binding, also the AP2 alpha binding to DNA may initiate transcriptional silencing by recruiting of DNMTs [40, 41]. Therefore it is possible that the methylation of binding sites Sp1, AP2 alpha and E2F located in the two more densely methylated regions of VAV3 promoter can inhibit its binding and its subsequent transcriptional activation. This event could explain the expression decrease of the VAV3 gene in HaCaT cells with overexpression of DNMT3B.

The overexpression of DNMT3B in HaCaT cells, downregulates the expression of SORBS2 and GPR137 genes, but the methylation of its promoters do not increase. SORBS2 is a scaffold protein involved in the assembly of signaling complexes in stress fibers and actin cytoskeleton [42, 43]. This gene is considered as putative tumour suppressor and although there is evidence of the loss or decrease of its expression in cervical and pancreatic cancer [44, 45], there is no evidence that this is due to promoter methylation. GPR137 is an integral membrane protein that belongs to the GPR137 family of cell mediators of signal transduction [46, 47]. Although the role of GPR137 in cancer is little known, several reports indicate that this gene is important a regulator of cell growth, apoptosis, invasion and migration in different types of human cancer [48-52]. Similar to SORBS2 there are no reports of abnormal methylation of the GPR137 promoter in human cancer. It is therefore likely that additional events are causing the downregulation the expression of SORBS2 and GPR137 genes. For example, methylation-independent repressor activities of DNMT3B [53].

In the current study, we found overexpression of DNMT3B in cervical cancer and various cancer cell lines. This event has been previously reported in various types of human cancer [8, 9, 13]. We also reported overexpression of DNMT3B and low levels of VAV3, SORBS2 and GPR137 in cervical, lung and breast cancer cell

lines. This could indicate that the findings in the DNMT3B overexpression in HaCaT cells model also occur in primary human tumors and human cancer cell lines.

In conclusion, our results suggest that the overexpression of DNMT3B in HaCaT cells, modulate the expression of genes related to cancer, downregulate the expression of 151 genes with CpG islands and downregulate the expression of the VAV3 gene via methylation of its promoter. These findings highlight the importance of DNMT3B in the gene expression and human cancer.

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Disclosure of conflict of interest

None.

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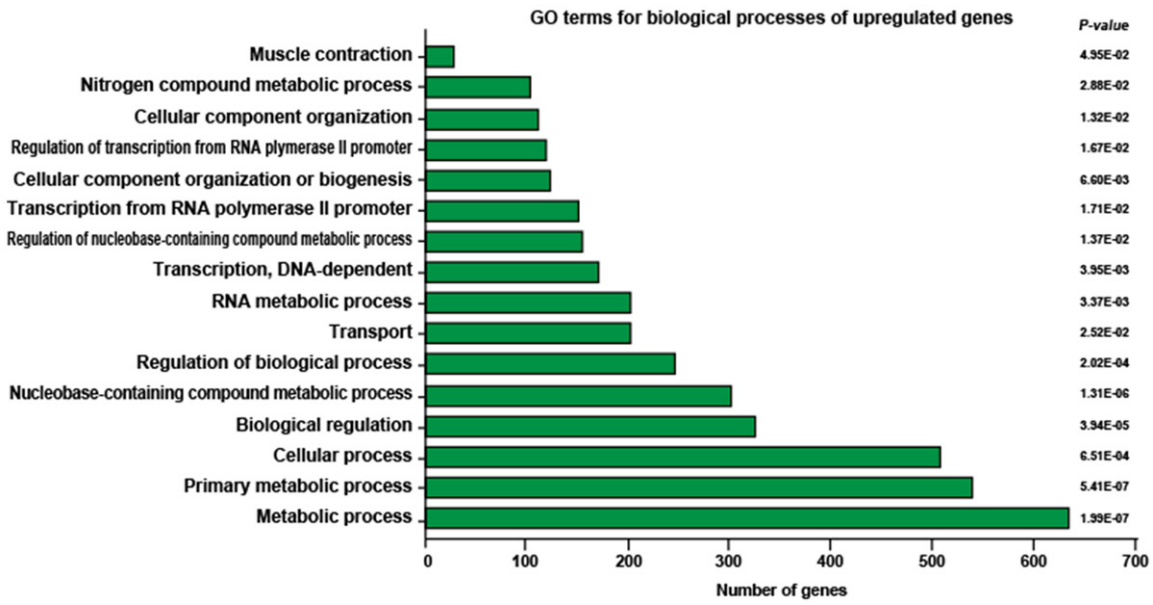


Supplementary Figure 1. Gene ontology (GO) analysis for upregulated genes by overexpression of DNMT3B in HaCaT cells.

Supplementary Table 1. Genes with CpG island downregulated by overexpression of DNMT3B in HaCaT cell

Gene-ID	Gene symbol	Gene name	Epigenetic evidence
ENSG00000106477	TSGA14	Centrosomal protein 41 kDa	Methylated in Ewing sarcoma (ES) cell lines and primary ES [1].
ENSG00000134215	VAV3	VAV3 guanine nucleotide exchange factor	Methylated in breast cancer cell lines [2].
ENSG00000196263	ZNF471	Zinc finger protein 471	Methylated in colorectal cancer [3].
ENSG00000163132	MSX1	Msh homeobox 1	Methylated in leukemia (T-ALL, T-linage leukemia) [4], and testicular cancer [5]. Furthermore, MSX1 is a repressor of cell cycle in human ovarian cancer cells [6]. Downregulated in cervical cancer tissue and cervical cell lines [7].
ENSG00000095002	MSH2	MutS homolog 2	Methylated in hepatocellular carcinoma [8], and Lynch Syndrome tumors [9].
ENSG00000165671	NSD1	Nuclear receptor binding SET domain protein 1	Methylated in human neuroblastoma and glioma cells [10].
ENSG00000170558	CDH2	Cadherin 2, type 1, N-cadherin	Methylated in primary gastric cancer, gastric cancer cell lines [11], and colon cancer [12].
ENSG00000112541	PDE10A	Phosphodiesterase 10A	Methylated in colorectal cancer [13].
ENSG00000136158	SPRY2	Sprout RTK signaling antagonist 2	Methylated in invasive prostate cancer cell lines (CaP) [14].
ENSG00000197579	TOPORS	Topoisomerase I binding, arginine/serine-rich E3, ubiquitin protein ligase	Methylated in colon adenocarcinoma [15].
ENSG00000183044	ABAT	4-aminobutyrate aminotransferase	Methylated in myelodysplastic syndrome [16], and glioblastoma [17].
ENSG00000162496	DHRS3	Dehydrogenase/reductase (SDR family) member 3	Methylated in neuroblastoma [18], and melanoma cell lines [19].
ENSG00000165325	CCDC67	Coiled-coil domain containing 67	Methylated in gastric cancer [20].
ENSG00000134202	GSTM3	Glutathione S-transferase mu 3	Methylated in Barrett's adenocarcinoma (BACs) samples [21].
ENSG00000116667	C1orf21	Chromosome 1 open reading frame 21	Methylated in squamous cell carcinoma (SCC) [22].
ENSG00000137962	ARHGAP29	Rho GTPase activating protein 29	Methylated in mantle cell lymphomas (MCL) cell lines and primary MCL samples [23].
ENSG00000147889	CDKN2A	Cyclin-dependent kinase inhibitor 2A	Methylated in cervical cancer [24, 25], in patients with non-invasive urinary bladder [26].
ENSG00000108753	TCF2	HNF1B homeobox B	Methylated in ovarian cancer cell lines and primary ovarian cancers [27].
ENSG00000116754	SFRS11	Serine/arginine-rich splicing factor 11	Xenoestrogen bisphenol A (BPA) induce methylation of SFRS11 gene in human breast epithelial cells [28].
ENSG00000172175	MALT1	MALT1 paracaspase	Methylated in oral carcinoma [29].
ENSG00000113569	NUP155	Nucleoporin 155 kDa	Methylation of NUP155 gene has been associated with breast cancer risk and is considered an epimarker in this type of cancer [30].
ENSG00000136114	THSD1	Thrombospondin, type I, domain containing 1	Methylated in colorectal cancer [31], and esophageal squamous cell carcinoma (ESCCC) [32].
ENSG00000159346	ADIPOR1	Adiponectin receptor 1	Methylated in overweight children [33].
ENSG00000163702	IL17RC	Interleukin 17 receptor C	Hipomethylated in age related macular degeneration (AMD) patients; therefore, suggesting that the DNA methylation pattern and expression of IL17RC may potentially serve as a biomarker for diagnosis of AMD [34].
ENSG00000143194	MAEL	Maelstrom spermatogenic transposon silencer	Hypomethylated in colorectal cancer [35].
Gene-ID	Gene symbol	Gene	Tumor suppressor evidence
ENSG00000107968	MAP3K8	Mitogen-activated protein kinase kinase kinase 8	Is a tumor suppressor in lung cancer [36].
ENSG00000125347	IRF1	Interferon regulatory factor 1	IRF1 acts as a tumor suppressor in breast cancer [37].
ENSG00000070731	ST6GALNAC2	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	ST6GALNAC2 acts as a breast cancer metastasis suppressor [38].
ENSG00000136158	SPRY2	Sprout RTK signaling antagonist 2	Is proposed as a potential tumor suppressor in prostate cancer [39].
ENSG00000197579	TOPORS	Topoisomerase I binding, arginine/serine-rich E3, ubiquitin protein ligase	Is possibly a tumor suppressor in colon adenocarcinoma [15, 40].
ENSG00000165325	CCDC67	Coiled-coil domain containing 67	Is a putative tumor suppressor gene in gastric cancer [20].

ENSG00000137962	ARHGAP29	Rho GTPase activating protein 29	Is a novel candidate tumor suppressor in mantle cell lymphomas [23].
ENSG00000136114	THSD1	Thrombospondin, type I, domain containing 1	Is considered a candidate tumor suppressor in esophageal squamous cell carcinoma [32].
ENSG00000080839	RBL1	Retinoblastoma-like 1	RBL1 or p70 can suppress the cell growth in Saos-2 and C-33A cells. The growth suppression effect of p70 is cell-type and cell-cycle stage dependent [41]; on the other hand, RBL1 is downregulated in gliomas and it can act as tumor suppressor [42].
Gen-ID	Gene symbol	Gene name	Cancer involvement
ENSG00000173068	BNC2	Basonuclin 2	Lower BNC2 expression has been demonstrated in epithelial ovarian cancer (EOC) cell cultures compared to normal ovarian cell lines [43]. BNC2 is a known EOC susceptibility gene. Future studies should further explore the role of DNA methylation in BNC2 [44].
ENSG00000112499	SLC22A2	Solute carrier family 22	Downregulated in pancreatic cancer [45]. High levels of OCT2 (SLC22A2) indicate severe invasion, but also better prognosis in metastatic colorectal cancer (mCRC) patients treated with oxaliplatin-based chemotherapy, possibly because of its role in oxaliplatin susceptibility [46].
ENSG00000135678	CPM	Carboxypeptidase M	Carboxypeptidase M is not expressed in human renal cell carcinoma tumor cells [47].
ENSG00000184979	USP18	Ubiquitin specific peptidase 18	Decreased expression of USP18 is a reliable prognostic marker for cancer specific survival in muscle invasive bladder cancer (MIBC) [48].
ENSG00000169398	PTK2	Protein tyrosine kinase 2	Is expressed in several human malignancies (Sulzmaier et al., 2014; Zhao et al., 2009), as well as cervical cancer [49].
ENSG00000141570	CBX8	Chromobox homolog 8	Is a novel oncogene that promotes the proliferation of tumor cells and raises the resistance of neoplasms to chemotherapy in esophageal carcinoma [50].
ENSG00000065361	ERBB3	Erb-b2 receptor tyrosine kinase 3	ROS inducing ERBB3 expression in OVCAR-3 cells [51].
ENSG00000124782	RREB1	Ras responsive element binding protein 1	Is overexpressed in colorectal adenocarcinoma tumors and cell lines [52], and prostate cancer [53].
ENSG00000006634	DBF4	Protein DBF4 homolog (Activator of S phase Kinase)	Highly expressed in many cancer cell lines [54].
ENSG00000157764	BRAF	B-Raf proto-oncogene, serine/threonine kinase	Mutations in BRAF is a frequent event in colorectal cancers (CRC) and BRAF mutations are associated with methylator phenotype in CRC [55-57]. Overexpressed in breast brain metastases [58].
ENSG00000197694	SPTAN1	Spectrin, alpha, non-erythrocytic 1	Linked with tumor progression and ovarian malignancy [59].
ENSG00000135823	STX6	Syntaxin 6	Overexpressed in human cancer as well as, breast, colon, pancreatic, prostate, bladder, skin, testicular, tongue, cervical, liver, lung and gastric cancer and has a role in cellular migration [60].
ENSG00000198682	PAPSS2	3,-phosphoadenosine 5,-phosphosulfate synthase 2	Expressed in ER-positive breast cancer tissues [61].
ENSG00000146242	TPBG	Trophoblast glycoprotein	Expressed in colorectal carcinoma [62], bladder, breast, cervix, endometrium, lung, esophagus, ovary, pancreas, stomach carcinomas [63].
ENSG00000125755	SYMPK	Symplekin	Expressed in human colorectal cancer and promotes tumorigenesis [64].
ENSG00000118898	PPL	Periplakin	Is highly expressed in triple-negative breast cancer (TNBC) [65].
ENSG00000165030	NFIL3	Nuclear factor, interleukin 3 regulated	Highly expressed in basal-like breast cancer and glioblastoma multiforme and NFIL3 expression is strongly correlated with poor prognosis in breast cancer [66].
ENSG00000112473	SLC39A7	Solute carrier family 39 (zinc transporter), member 7	MCF7 cell models of acquired tamoxifen resistance (TamR cells) have increased levels of zinc and zinc transporter, resulting in an enhanced response to exogenous zinc, leading to increased growth and invasion [67].
ENSG00000064042	NP_055803.1 (LIMCH1)	LIM and calponin homology domains 1	Overexpressed in ERα-positive breast tumors with PIK3CA mutations [68].
ENSG00000082996	RNF13	Ring finger protein 13	RNF13 gene expression is associated with cancer development [69]. Furthermore, RINF13 is overexpressed in pancreatic cancer [70].
ENSG00000101182	PSMA7	Proteasome (prosome, macropain) subunit, alpha type, 7	Reduced expression in prostate cancer [71]. PSMA7 inhibits the proliferation, tumorigenicity and invasion of A549 human lung adenocarcinoma cells in vitro [72], and PSMA is highly expressed in colorectal cancer cell lines [73].

ENSG00000151240	DIP2C	Disco-interacting protein 2 homolog C	Somatic mutations in the DIP2C gene have an impact on protein function in breast cancer [74].
ENSG00000136986	DERL1	Derlin 1	Overexpressed in breast-brain metastases [75].
ENSG00000107077	JMJD2C	Lysine (K)-specific demethylase 4C	Overexpressed in colon cancer cell lines and confers a pro-growth effect on colon cancer cells [76].
ENSG00000173264	GPR137	G protein-coupled receptor 137	GPR137 is highly expressed in multiple human gastric cancer cell lines; however, Its role in human disease onset has remained to be elucidated [77].
ENSG00000173890	GPR160	G protein-coupled receptor 160	G protein-coupled receptor 160 (GPR160) has been proposed as an oncogene involved in nasopharyngeal carcinoma [78].
ENSG00000070886	EPHA8	EPH receptor A8	mRNA expression in colon cancer [79].
ENSG00000136807	CDK9	Cyclin-dependent kinase 9	Is required for the proliferation of HCC cell lines [80]; furthermore, CDK9 is important for cancer cell survival [81].
ENSG00000150630	VEGFC	Vascular endhothelial growth factor C	Overexpression of VEGFC in breast cancer cells promotes metastasis to lymph nodes and lungs [82]; furthermore expression of VEGFC has been reported in various types of cancer such as breast, lung, squamous cell, sarcomas, melanomas [83], mesothelioma [84]; gastric [85] and other.
ENSG00000138685	FGF2	Fibroblast growth factor 2	Plays an important role in prostate cancer [86], lung [87], and head and neck [88].
ENSG00000158711	ELK4	ETS-like transcription factor 4 (ELK4)	Expressed in prostate cancer and contributes to cellular growth [89, 90].
ENSG00000168438	CDC40	Cell division cycle 40	Upregulated in the primary CRC tissues, and promotes CRC cell growth [91].
ENSG00000117298	ECE1	Endothelin converting enzyme 1	Expressed in human prostate cancer cell lines [92], and ovarian carcinoma cells [93]. ECE-1 contributes to invasion and migration in cancer [94, 95].
ENSG00000105647	PIK3R2	Phosphoinositide-3-kinase, regulatory subunit 2 (beta)	PIK3R2 mutations have been reported in endometrial tumors and PIK3R2 is considered a novel endometrial cancer gene [96] .
ENSG00000127914	AKAP9	A kinase (PRKA) anchor protein 9	The AKAP9 M463I T allele is associated with an increased breast cancer risk in familial breast cancer [97].
ENSG00000118733	OLFM3	Olfactomedin 3	Plays an important role in anoikis resistance, and OLFM3 is expressed in lung, breast and resistant nasal cancer cell lines anoikis [97].
ENSG00000129515	SNX6	Sorting nexin 6	Sorting nexin 6 (SNX6) interacts with breast cancer metastasis suppressor 1 (BRMS 1) protein and favoring transcriptional repression, furthermore, BRMS1-SNX6-HDAC complex may modulate the transcriptional repression [98].
ENSG00000109182	NP_079363.1 (CWH43)	Cell wall biogenesis 43 C-terminal homolog	Cell Wall Biogenesis 43 C-Terminal Homolog (CWH43) is downregulated in colorectal tumor tissues, but its role in colorectal cancer has not been reported [99].
ENSG00000185250	PPIL6	Peptidylprolyl isomerase (cyclophilin)-like 6	Is a novel gene identified in genomic aberrations associated with prostate cancer progression, but its function has not been characterized [100].
ENSG00000175054	ATR	ATR serine/threonine kinase	Human colorectal cancer cells require Ataxia telangiectasia mutated and Rad3-related (ATR) for cell cycle progression after IR treatment [101]; ATR is a therapeutic target in cancer [102, 103]; ATR mutations in endometrial cancer are associated with reduced overall survival and disease-free survival [104].
ENSG00000075388	FGF4	Fibroblast growth factor 4	Exogenous FGF4 provides an advantage in cell growth and tumorigenicity of HBL100 and MCF7 breast cancer cells and the cells that expressed FGF4 show an aggressive phenotype, actually, spontaneous metastasis [105-108].
ENSG00000125304	TM9SF2	Transmembrane 9 superfamily member 2	Expressed in breast cancer cells, and it is propose as a diagnostic biomarker [109]; the expression of TM9SF2 in colorectal cancer (CRC) patients has been associated with poor survival [110].
ENSG00000072274	TFRC	Transferrin receptor	Expressed in human pancreatic cancer and in neuroendocrine carcinoma of pancreas and it has been proposed as a marker of malignant transformation [111]; furthermore, TFRC is expressed in esophageal squamous cell carcinoma (ESCC), and it can be a prognostic factor in patients with ESCC [112]. TFRC is upregulated in invasive cervical cancer and it is associated with invasion in this type of cancer [113].
ENSG00000141642	ELAC1	ElaC ribonuclease Z 1	Downregulated in colorectal liver metastases [114].
ENSG00000072042	RDH11	Retinol dehydrogenase 11 (all-trans/9-cis/11-cis)	Retinol dehydrogenase 11 (RDH11 or PSDR1) is overexpressed in prostate cancer and it has been suggested that it may play role in prostate carcinoma [115, 116].

ENSG00000180667	YOD1	YOD1 deubiquitinase	YOD1 was identified as a target of miR-373 in cervical cancer, however, the role of YOD1 in cancer has not yet been elucidate [117].
ENSG00000173253	DMRT2	Doublesex and mab-3 related transcription factor 2	DMRT2 is a transcription factor that is downregulated in clear cell renal cell carcinoma (ccRCC) [118].
ENSG00000101856	PGRMC1	Progesterone receptor membrane component 1	Plays a role in cell growth, cell viability and chemoresistance in endometrial tumors, ovarian cancer, and uterine sarcoma [119-121]; furthermore, this gene is associated with tumorigenesis in lung cancer [122].
ENSG00000141985	SH3GL1	SH3-domain GRB2-like 1	Expressed in human medulloblastoma (MB) cell lines and is a target of miR-128 [123].
ENSG00000173141	MRP63	Mitochondrial ribosomal protein L57	Downregulated in glioma cell lines with 13q deletion [124].
ENSG00000138709	LARP2	The ribonucleoprotein domain family, member 1B	Expressed in meningiomas [125].
ENSG00000177189	RPS6KA3	Ribosomal protein S6 kinase, 90 kDa, polypeptide 3	RPS6KA3 is frequently mutated in hepatocellular carcinoma (HCC) [126].
ENSG00000164270	HTR4	5-hydroxytryptamine (serotonin) receptor 4, G protein-coupled	Overexpressed in high grade tumours and DU145 and LNCap prostate cancer [127].
ENSG00000122679	RAMP3	Receptor (G protein-coupled) activity modifying protein 3	Expressed in prostate cancer tissue and might be involved in tumor cell growth [128].
ENSG00000186017	ZNF566	Zinc finger protein 566	Zinc finger proteins (ZNF) are implicated in the development of various types of cancer [129-134].
ENSG00000198522	ZNF512	Zinc finger protein 512	
ENSG00000171467	ZNF318	Zinc finger protein 318	
ENSG00000135502	SLC26A10	Solute carrier family 26, member 10	The solute carriers (SLC) transporters expressed in cancer cells promoting cell growth and SLC members are associated with cancer therapy [135, 136].
ENSG00000075415	SLC25A3	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3	
ENSG00000163848	SLC12A8	Solute carrier family 12, member 8	

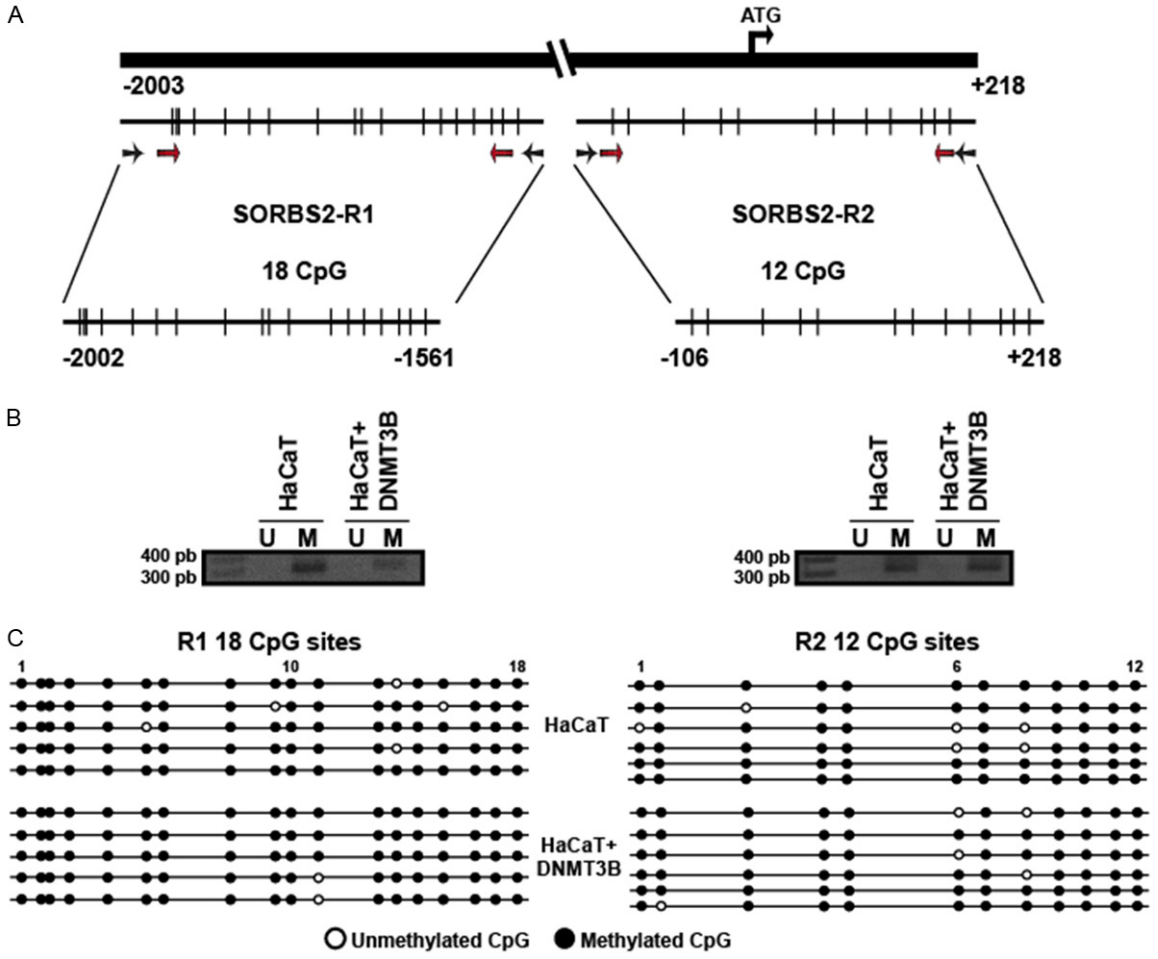
Gene-ID	Gene symbol	Name gene	Cancer information not available
ENSG00000100767	PAPLN	Papillin, proteoglycan-like sulfated glycoprotein	
ENSG00000138032	PPM1B	Protein phosphatase, Mg2+/Mn2+ dependent, 1B	
ENSG00000141198	TOM1L1	Target of myb1 like 1 membrane trafficking protein	
ENSG00000125534	C20orf149	Pancreatic progenitor cell differentiation and proliferation factor	
ENSG00000022277	C20orf43	Replication termination factor 2 domain containing 1	
ENSG00000121931	C1orf103	Ligand dependent nuclear receptor interacting factor 1	
ENSG00000120685	C13orf23	Proline and serine rich 1	
ENSG00000103254	C16orf24	Family with sequence similarity 173, member A	
ENSG00000001460	C1orf201	Sperm-tail PG-rich repeat containing 1	
ENSG00000175707	C1orf172	Keratinocyte differentiation factor 1	
ENSG00000168175	C14orf32	Mitogen-activated protein kinase 1 interacting protein 1-like	
ENSG00000166262	C15orf33	Family with sequence similarity 227, member B	
ENSG00000185567	Q5BKX7_HUMAN (C14orf78)	AHNAK nucleoprotein 2	
ENSG00000100625	SIX4	SIX homeobox 4	
ENSG00000111725	PRKAB1	Protein kinase, AMP-activated, beta 1 non-catalytic subunit	
ENSG00000166965	RCCD1	RCC1 domain containing 1	
ENSG00000153951	OR4D2	Olfactory receptor, family 4, subfamily D, member 2	
ENSG00000164366	CCDC127	Coiled-coil domain containing 127	
ENSG00000143630	HCN3	Hyperpolarization activated cyclic nucleotide gated potassium channel 3	

ENSG00000023909	GCLM	Glutamate-cysteine ligase, modifier subunit
ENSG00000087470	DNM1L	Dynamin 1-like
ENSG00000162188	GNG3	Guanine nucleotide binding protein (G protein), gamma 3
ENSG00000168268	NT5DC2	5,-nucleotidase domain containing 2
ENSG00000167700	MFSD3	Major facilitator superfamily domain containing 3
ENSG00000183340	JRKL	JRK-like
ENSG00000174740	PABPC5	Poly (A) binding protein, cytoplasmatic 5
ENSG00000052723	NP_079349.1 (SIKE1)	Suppressor of IKBKE 1
ENSG00000054116	TRAPPC3	Trafficking protein particle complex 3
ENSG00000138073	PREB	Prolactin regulatory element binding
ENSG00000171763	SPATA5L1	Spermatogenesis associated 5-like 1
ENSG00000112972	HMGCS1	3-hydroxy-3-methylglutaryl-CoA synthase 1
ENSG00000112992	NNT	Nicotinamide nucleotide transhydrogenase
ENSG00000141994	DUS3L	Dihydrouridine synthase 3-like
ENSG00000089775	ZBTB25	Zinc finger and BTB domain containing 25
ENSG00000123737	EXOSC9	Exosome component 9
ENSG00000068724	TTC7A	Tetratricopeptide repeat domain 7A
ENSG00000138363	ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyl-transferase/IMP cyclohydrolase
ENSG00000159202	UBE2Z	Ubiquitin-conjugating enzyme E2Z
ENSG00000171861	RNMTL1	RNA methyltransferase like 1
ENSG00000127824	TUBA1	Tubulin, alpha 4a
ENSG00000157212	PAXIP1	PSX interacting (with transcription-activation domain) protein 1
ENSG00000084734	GCKR	Glucokinase (hexokinase 4) regulator
ENSG00000166337	TAF10	TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 30 kDa
ENSG00000149256	ODZ4	Teneurin transmembrane protein 4
ENSG00000004777	SNX26	Rho GTPase activating protein 33
ENSG00000113811	SELK_HUMAN	Selenoprotein K
ENSG00000165678	GHITM	Growth hormone inducible transmembrane protein
ENSG00000176261	ZBTB8OS	Zinc finger and BTB domain containing 8 opposite strand
ENSG00000163964	PIGX	Phosphatidylinositol glycan anchor biosynthesis, class X
ENSG00000138617	PARP16	Poly (ADP-ribose) polymerase family, member 16
ENSG00000066583	ISOC1	Isochorismatase domain containing 1
ENSG00000179562	GCC1	GRIP and coiled-coil domain containing 1
ENSG00000197568	HHLA3	HERV-H LTR-associating 3
ENSG00000132846	ZBED3	Zinc finger, BED-type containing 3
ENSG00000135241	PNPLA8	Patatin-like phospholipase domain containing 8

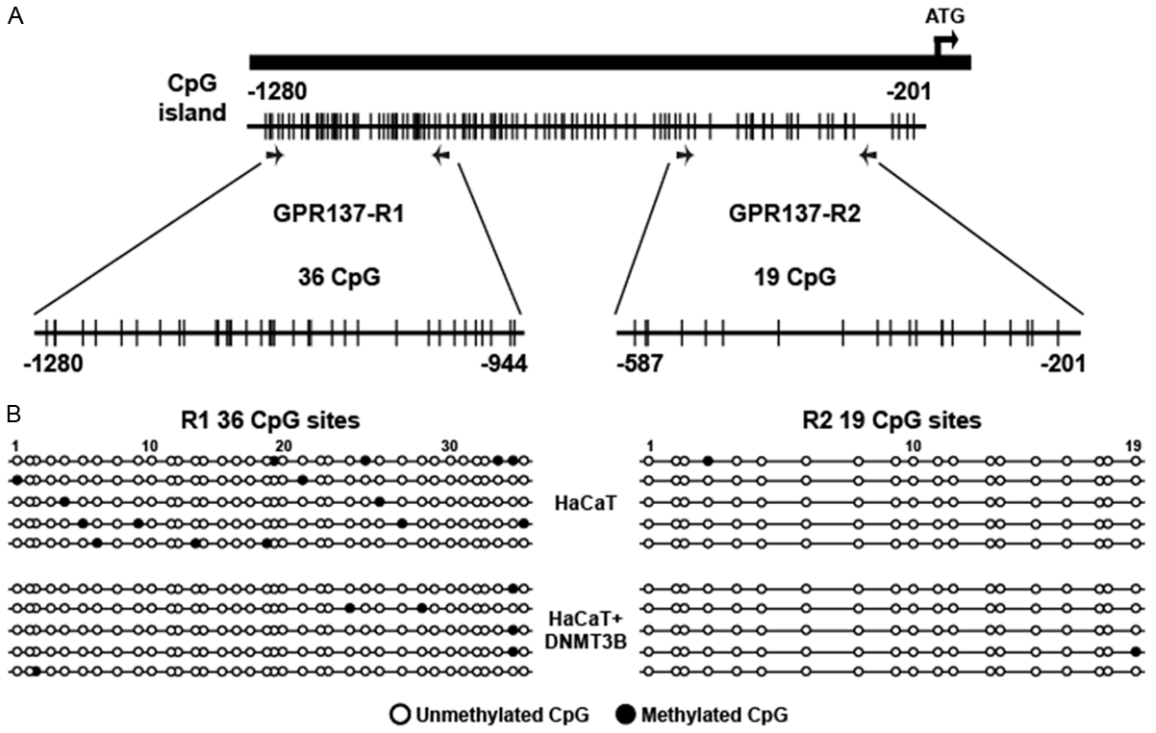
ENSG00000138439	ALS2CR13	family with sequence similarity 117, member B
ENSG00000178636	Q8N7N2_HUMAN	-
ENSG00000166451	CENPN	Centromere protein N
ENSG00000130363	RSHL2	Radial spoke 3 homolog (Chlamydomonas)
ENSG00000106012	IQCE	IQ motif containing E
ENSG00000166863	TAC3	Tachykinin 3
ENSG00000157890	MEGF11	Multiple EGF-like-domains 11

Supplementary Table 2. Genes selected for RT-qPCR validation and methylation analysis

Gene-ID	Gene symbol	Gene name	GO (Biological process)	Epigenetic evidence and cancer involvement
ENSG00000095002	MSH2	MutS homolog 2	Biological regulation Cellular component organization Metabolic process Reproduction Response to stimulus	Methylated in hepatocellular carcinoma [8], and Lynch Syndrome tumors [9].
ENSG00000165671	NSD1	Nuclear receptor binding SET domain protein 1	Cellular component organization Cellular process Metabolic process	Methylated in neuroblastoma and glioma [137].
ENSG00000134215	VAV3	VAV3 guanine nucleotide exchange factor	Cellular process Biological regulation Immune system process Metabolic process Multicellular organismal process Response to stimulus	Methylated in breast cancer cell lines [138].
ENSG00000163132	MSX1	Msh homeobox 1	Metabolic process Developmental process Biological regulation	Methylated in leukemia (T-ALL, T-linage leukemia) [4], and testicular cancer [5]. Furthermore, MSX1 is a repressor of cell cycle in human ovarian cancer cells [6]. Downregulated in cervical cancer tissue and cervical cell lines [7].
ENSG00000137962	ARHGAP29	Rho GTPase activating protein 29	Non-annotated gene	Methylated in mantle cell lymphoma [139].
ENSG00000136114	THSD1	Thrombospondin, type I, domain containing 1	Non-annotated gene	Methylated and candidate tumor suppressor gene in colon cancer [140].
ENSG00000070731	ST6GALNAC2	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	Metabolic process	Candidate tumor suppressor gene in breast cancer [141].
ENSG00000154556	SORBS2	Sorbin and SH3 domain containing 2	Metabolic process	Putative tumor suppressor gene involved in cervical carcinogenesis [142].
ENSG0000017326	GPR137	G protein-coupled receptor 137	Non-annotated gene	Highly expressed in multiple human gastric cancer cell lines; however, Its role in human disease has remained to be elucidated [77].
ENSG00000001460	C1ORF201	Sperm-tail PG-rich repeat containing 1	Non-annotated gene	No studies have report its relationship with human cancer but plays a role in apoptosis [143].



Supplementary Figure 2. Methylation analysis of SORBS2 promoter in HaCaT cells. A: Schematic representation of the CpG island and CpG sites in the SORBS2 promoter. For methylation analysis the SORBS2 promoter was divided into 2 regions: R1 -2002 to -1561 with 18 CpGs and R2 -106 to +218 with 12 CpGs, the positions are relative to the transcription start site. The primers for MSP and BSP are indicated by black and red arrows, respectively. Each CpG site is represented by a vertical bar. B: The methylation status of the SORBS2 promoter (R1 and R2) was determined by MSP in HaCaT cells with overexpression of DNMT3B and control HaCaT cells. U showed unmethylation-specific primer amplification, M showed methylation-specific primer amplification. C: BSP analysis of the SORBS2 promoter (R1 and R2) in HaCaT cells with overexpression of DNMT3B and control HaCaT cells. Black circles represent methylated CpG sites and white circles represent unmethylated CpG sites.



Supplementary Figure 3. Methylation analysis of GPR137 promoter in HaCaT cells. A: Schematic representation of the CpG island and CpG sites in the GPR137 promoter. For methylation analysis the GPR137 promoter was divided into 2 regions: R1 -1280 to -944 with 36 CpGs and R2 -587 to -201 with 19 CpGs, the positions are relative to the transcription start site. The primers for BSP are indicated by black arrows. Each CpG site is represented by a vertical bar. B: BSP analysis of the GPR137 promoter (R1 and R2) in HaCaT cells with overexpression of DNMT3B and control HaCaT cells. Black circles represent methylated CpG site and white circles represent unmethylated CpG site.

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