Intermediate- and low-methylation epigenotypes do not correspond to CpG island methylator phenotype (low and -zero) in colorectal cancer.

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Abstract

Background: Most recent genome-wide studies on the CpG island methylation in colorectal cancer (CRC) have led to the discovery of at least three distinct methylation clusters. However, there remains an uncertainty whether the CRC clusters identified in these studies represent compatible phenotypes.

Methods: We performed comprehensive genome-scale DNA methylation profiling by Illumina Infinium HumanMethylation27 of 21 DNA pools that represent 84 CRC samples divided according to their high-, intermediate-, and low-methylation epigenotypes (HME, IME, and LME, respectively) and 70 normal-adjacent colonic tissues. We have also examined the relationship between three epigenotypes and chromosomal gains and deletions (assessed by Comparative Genomic Hybridization) in a group of 100 CRC samples.

Results: HME subgroup showed features associated with CpG island methylator phenotype – high (CIMP-high) including methylation of specific CpG sites (CpGs) as well as significantly lower mean number of chromosomal imbalances when compared to other epigenotypes. IME subgroup displayed lowest number of methylated CpGs (717 versus 2399 and 2679 in HME and LME, respectively) and highest mean number of chromosomal imbalances when compared to HME ($p = 0.001$) and LME ($p = 0.004$). A comparison between the methylation profiles of three epigenotypes revealed more similarities between the HME and LME (1669 methylated CpGs overlapped) than HME and IME (673 methylated CpGs overlapped).

Conclusion: Our results provide evidence that IME and LME CRCs show opposite features to those that have been previously attributed to CIMP-low and CIMP-0 CRCs.

Impact: These discrepancies should be considered when interpreting the data from a particular epigenotyping method.

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Introduction

It is now well established that sporadic CRCs are characterized by significant heterogeneity with regard to global epigenomic status including CpG island methylation (1). The existence of distinct epigenotype in CRC refered to as CpG island methylator phenotype (CIMP) has been reported for the first time in 1999 (2). Since then, a CIMP-specific marker panel has been developed (Weisenberger's one-panel method) and further extended (Ogino's one-panel method) which allowed for comprehensive studies of CIMP (3, 4).

CIMP-high tumors have been associated with distinct molecular and clinical characteristics including high CpG islands methylation rates, older age, proximal tumor location, microsatellite instability (MSI), frequent *BRAF* V600E mutation and extensive methylation of the 3p22 region (5, 6). Subsequent studies by Ogino et al. have demonstrated the existence of tumors enriched for *KRAS* mutations that displayed less extensive methylation of CIMP-related markers (designated as CIMP-low) (7). Finally, tumors with infrequent CIMP-related marker methylation (designated as CIMP-0) have been characterized by a frequent loss of heterozygosity at 18q and *TP53* mutations (8, 9).

Recent unsupervised hierarchical clustering of genome-wide selected marker panel (Yagi's two-panels method) has confirmed the existence of three distinct clusters in CRC referred to as the high-, intermediate-, and low-methylation epigenotypes (HME, IME, and LME, respectively) (10). However, it is not clearly established whether HME, IME and LME are equivalent to CIMP-high, CIMP-low and CIMP-0 (9, 11). Therefore, our study aimed at clarifying the issue of whether differences between these epigenotypes exist. Using genome-scale methylation profiling of 27,578 CpG sites (Illumina Infinium HumanMethylation27) we profiled

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pooled 84 CRC samples previously divided according to their HME/IME/LME epigenotypes (12). We further characterized chromosomal imbalances in 100 CRCs (also divided according to their epigenotype status) by means of comparative genomic hybridization (CGH). Finally, we related our findings to the very recent DNA methylation analysis of CIMP-high, CIMP-low and non-CIMP tumors .

Our results demonstrate that IME CRCs show relatively low methylation frequency of CpG sites and a high mean number of chromosomal imbalances when compared to HME and LME CRCs. HME and LME subclasses display more related pattern of DNA methylation and level of chromosomal imbalances when compared to IME CRCs.

Materials and Methods

Human CRC tissue samples and human CRC cell lines. Surgically resected frozen tissues of 233 colorectal cancers and matched normal colon samples were obtained from the 2nd Department of General and Oncological Surgery, Wroclaw Medical University, Wroclaw and the 1st Department of Surgical Oncology, Lower Silesian Oncology Center, Wroclaw. The CRC patient group consisted entirely of Polish individuals (all Caucasians). Only patients with primary, sporadic colorectal cancer who had not received preoperative therapy were included in the study. Informed consent was obtained from all the patients. The study was accepted by the Wroclaw Medical University Ethics Committee.

DLD1 and SW480 cell lines were obtained from the International Institute of Molecular and Cell Biology, Laboratory of Cell Biology (Warsaw, Poland). HTC116, HT29, CACO-2 and LOVO were obtained from the Institute of Human Genetics, Division of Molecular Genetics (Tübingen, Germany). The cell lines were authenticated by using the panel of 11 STRs (AmpFlSTR SGM Plus, Applied

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Biosystems, US). The number of repeats for each locus was verified in CLIMA database (13).

Tumour samples with at least 40% viable neoplastic cell content were used for DNA isolation using the Gentra Puregene Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's manual. Prior pooling and CGH study DNA quality was verified by NanoDrop measurements, visual inscpection of electrophoretic gel and β-globin gene amplification (14).

Bisulfite modification and epigenotyping. Bisulfite treatment of 1 µg genomic DNA obtained from resected frozen tissues and cell lines was carried out using the EpiTect kit (Qiagen, Hilden, Germany). Epigenotyping was performed by a combined bisulfite restriction assay (COBRA) as previously described (12). We quantified DNA methylation in 7 specific promoters originally described by Yagi et al. (10). Briefly, after the analysis of the methylation of a panel of three markers (*CACNA1G*, *SLC30A10*, and *LOX*), HME tumors were defined as those with at least two methylated markers. The remaining tumors were screened using five markers (*SLC30A10*, *ELMO1*, *FBN2*, *THBD*, and *HAND1*), IME tumors were defined as those with at least three methylated markers. Tumors not classified as HME or IME were designated LME. Primer sequences were previously described (12).

Analysis of other molecular classifiers. Detection of *BRAF* V600E in tumor tissues was carried out using the mutant allele-specific PCR amplification described by Sapio et al. (15). Mutations at codon 12 of the *KRAS* gene were detected by PCR–RFLP as described by Miranda et al. (16). Mutations at codon 13 of the *KRAS* were assesed by SNaPshot minisequencing (primer sequences on request). Microsatellite instability

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(MSI) was determined by pentaplex PCR, using the quasimonomorphic markers BAT-26, BAT-25, NR-21, NR-22, and NR-24, as described in detail by Buhard et al. (17).

Samples selection and pooling prior microarray analysis. Samples were selected for microarray analysis on the base of DNA quality and epigenotype. After epigenotyping of 233 CRCs a great care was taken during samples selection to avoid disturbance in distribution of clinical and molecular features specific for HME/IME/LME epigenotypes. Finally, 20 HME, 32 IME, 32 LME CRCs and DNA extracted from 70 normal colon tissues (adjacent to 10 HME, 30 IME and 30 LME tumors) were selected for subsequent experiments (Table 1). DNA pools were constructed according to guidelines described by Docherty et al. (18, 19). Six biologically independent DNA pools were designed for each CRC epigenotype. Normal colon tissue was represented by 3 independent DNA pools. All 21 pools were verified for the epigenotype status by combined bisulfite restriction assay (COBRA) (see Supplemental Table 1 for the details).

DNA methylation mircoarray analysis. Bisulfite treatment of DNA pools was carried out using the EZ DNA Methylation Kit (Zymo Research, Orange, CA, USA) according to the manufacturer's protocol. The Infinium Methylation-27 Assay was used to detect methylation at 27,578 CpG sites, spanning 14,495 genes (20). After bisulfite conversion, each sample was whole-genome amplified (WGA) and enzymatically fragmented. The bisulfite-converted WGA-DNA samples were purified and applied to the BeadChips. Allele-specific primer annealing was followed by single-base extension using DNP- and Biotin-labeled ddNTPs. After hybridization, staining and washing the intensities of the individual beads were scanned in an Illumina Bead Array Reader. Subsequently quality control and statistical analysis was

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performed using various Bioconductor packages (v.2.10.0) (21). To assess quality of the arrays the signal intensities of control probes together with additional parameters (signal distributions of intensities, average methylation values, coefficient of variation of replicate beads, sactterplots) were analyzed. Taken together, all arrays were of good quality and were included in the subsequent data analysis. The data were normalized using „shift and scaling normalization" (22). 3981 previously reported, polymorphisms-containing or nonspecific probes were masked prior to the statistical analysis (23). The intensities of methylated and non methylated probes were used to calculate the methylation level (β-values) and the log ratio of the intensities (M-values). To find differentially methylated probes the M-values were used to calculate the coefficients of the linear model, which describe the methylation profile of the corresponding locus and the p-values were extracted (21), (24). Subsequently, the p-values were corrected for multiple testing using the Benjamini-Hochberg false discovery rate. All probes with difference in β - values ≥ 0.20 and $p \leq 0.05$ were retained (20). All Infinium DNA methylation data are deposited at the NCBI Gene Expression Omnibus under accession number GSE37740.

Samples selection and comparative genomic hybridization (CGH). Samples were selected for CGH on the base of epigenotype, DNA quality and availability. Eventually, 20 HME, 40 IME and 40 LME CRCs were selected for subsequent analysis (Table 2). CGH was performed as described by Kallioniemi et al. with minor modifications described by Tonnies et al. (25, 26). The telomeric, peri-centromeric, heterochromatic regions, as well as chromosomes X and Y were excluded from analysis. Chromosomal alterations of each sample were examined in 10 metaphases. To verify the results 23 cases were analyzed by reverse labelling. The

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Mann-Whitney test was used to compare the mean number of gains in different CRC subgroups.

Results

Genome-wide methylation analysis of three epigenotypes. We performed a comprehensive DNA methylation profiling of 21 DNA pools consisted of 84 CRC samples divided previously according to their epigenotypes (HME, IME and LME) and 70 normal-adjacent colon tissue by the use of Illumina Infinium HumanMethylation27 DNA assay (12). The characteristics of the selected samples are shown in Table 1. After exclusion of polymorphism-containing and nonspecific probes methylation status of 23,837 CpG sites was compared between each of CRC epigenotype and normal-adiacent tissue (23). We retained for further analysis only the CpG sites (CpGs) that displayed a difference in β – values of more than 0.20 and p – value $\leq$ 0.05 (after correction for multiple testing) (20). Using these criteria, we identified 3421 CpGs differentially methylated between all CRC epigenotypes and normal colonic tissues. The DNA methylation pattern of these CpGs was further investigated by principal components analysis (PCA). As shown in Figure 1A, HME, IME and LME epigenotypes clearly show distinct methylation profiles. The comparison of differentially methylated CpGs (Figure 1B) revealed that the HME and LME displayed comparable number of differentially methylated CpGs (2399 and 2679, respectively), whereas IME showed a lower total number of differentially methylated sites (n=717) when compared to the other epigenotypes. Substantial overlap (49% of all differentially methylated CpGs) between HME and LME was

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observed whereas HME and IME subgroups displayed the simultaneous methylation of 19% of all differentially methylated CpGs (Figure 1B).

Using data gathered in genome-wide methylation analysis we explored the methylation of 10 CpGs (*B3GAT2*, *FOXL2*, *KCNK13*, *RAB31*, *SLIT1, FAM78A, FSTL1, KCNC1, MYOCD,* and *SLC6A4*) that have been recently proposed by Hinoue et al. as a new "two-panel method" to differentiate CIMP-high and CIMP-low subtypes (9). Using this approach, HME subgroup was classified as CIMP-high whereas IME and LME subgroups as CIMP-0 and CIMP-low, respectively (Supplemental Table 2). Similar classification of HME, IME, LME, based on Infinium DNA methylation data, was obtained when we looked at the methylation of CIMP-specific markers (*CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, *SOCS1*, *CDKN2A*, *CRABP1*, *MLH1*) previously proposed by Ogino et al. (Supplemental Table 3) (4). Finally, the epigenotyping proposed by Yagi et al. could not be fully explored using our Infinium DNA methylation data, because of lack of probe for one of the HME/IME markers (*SLC30A10*) on Infinium HumanMethylation27 array (Supplemental Table 4) (10). Detailed array data for all above mentioned markers containing β – values and detection p-values for all 21 pools can be found in Supplemental Table 5.

Analysis of chromosomal imbalances in three epigenotypes. To assess the mean number of chromosomal imbalances in each of epigenotype we performed comparative genomic hybridization (CGH) using selected 20 HME, 40 IME and 40 LME CRCs (Table 2, see Supplementary CGH data for details) (12). IME showed a clearly higher mean number of total chromosomal imbalances (10.4) compared to the other CRC subtypes (4.5 in HME (p = 0.001) and 6.25 in LME (p = 0.004)). Next, we compared mean number of amplifications and deletions between epigenotypes. In relation to HME and LME mean number of amplifications was highest among IME

CRCs (1.95 and 3.45 versus 4.75, respectively). A similar observation was made in the context of deletions where IME displayed on average twice as many deletions (5.7) as in HME (2.5) and LME (2.8). The difference between HME and LME were much less pronounced and manifest itself via borderline difference in the mean number of amplifications (p = 0.045).

Epigenotyping of CRC cell lines by combined bisulfite restriction assay (COBRA). We epigenotyped six CRC cell lines (DLD1, HTC116, HT29, CaCo2, SW480 and LOVO) that have been characterized previously according to their CIMP status and/or epigenotype (HME, IME or LME) status (10, 27). DLD1, HTC116, HT29 and LOVO were classified as HME whereas CaCo2 and SW480 as IME (Figure 2, Table 3). The methylation level of each marker in each cell line is presented in the Supplemental Table 6.

Discussion

The methylator phenotype (CIMP) in CRC was identified by Toyota et al., after selecting particular CpG islands (CpGis) that displayed methylation in cancer-specific manner (so called MINT loci) (2). This allowed to distinguish subgroups of CRCs with prominent number of methylated CpGis, specific molecular features (*BRAF* mutation, MSI) and preferential localization in the proximal colon (later called CIMP-high or CIMP1) (1, 2). Subsequently, after screening methylation of 92 CpGis in 187 CRCs a new CIMP-specific marker panel was proposed by Weisenberger et al., and further enriched with new markers by Ogino et al (3, 4). Parallel to the searches for marker candidates, a subgroup of CRCs with less extensive CpGis methylation enriched for the *KRAS* mutants was revealed (CIMP-low or CIMP2) (7, 28). Finally, non-CIMP tumors (CIMP-0) have been correlated with a frequent loss of heterozygosity (LOH) at 18q that is regarded as one of the signs of chromosomal instability in CRC (8).

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Subsequent studies have focused on genome-wide searches for methylation markers suitable to distinguish between CIMP-low and CIMP-0 (9, 10). On the base of methylated DNA immunoprecipitation (MeDIP) and re-expression studies performed on two CRC cell lines (HTC116 and SW480) Yagi et al. recently proposed marker panel which led to classification of CRC cases into three distinct epigenotypes: HME, IME, and LME (10). Application of this marker panel and combined bisulfite restriction analysis (COBRA) has recently enabled us to accurately epigenotype 233 CRC cases (12).

Although, at least three marker panels (described by Ogino et al., Shen et al. and Yagi et al.) for CRC epigenotyping are currently in use there is no consensus whether clustered tumors represent comparable phenotypes with exception of the CIMP-high subgroup (4, 9, 10, 28). Indeed, attributes of CIMP-high/CIMP1/HME tumors (MSI, *BRAF* V600E, proximal localization and older age) seem to be constant across the number of studies (1). In contrast, some previous studies had implied that there is no certainty whether IME/CIMP2/CIMP-low are equivalent presumably because of limited genetic and clinical features supporting the existence of this subtype (9). One recent genome-scale DNA methylation study provided methylation profiles of CIMP-high, CIMP-low and non-CIMP subgroups using the Illumina Infinium DNA methylation assay comprised of 27,578 CpG sites (9). These data prompted us to examine whether pooled HME, IME and LME CRCs will display comparable methylation profiles by using an identical DNA methylation platform. Surprisingly, IME subgroup displayed lowest number of methylated CpGs when compared to HME and LME. Moderate CpGis methylation frequencies were shown by Hinoue et al. as a feature of non-CIMP tumors whereas CIMP-low was the second most methylated subgroup of tumors based on clustering analyzes (9). Further, we demonstrated a

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substantial overlap between HME and LME-associated markers (Figure 1B). Such relation had been previously reported for CIMP-high and CIMP-low subgroups (9). By the use of CIMP-high and CIMP-low defining markers (described by Hinoue et al.), we classified HME subgroup as CIMP-high whereas IME and LME subgroups as CIMP-0 and CIMP-low, respectively (Supplemental Table 2) (9). Similar classification was achieved when we applied markers described by Ogino et al. (Supplemental Table 3) (4). We admit that methylation of IME-specific Yagi's markers in IME/LME pools displayed by Infinium DNA methylation assay (Supplemental Table 4 and 5) is not as clear as methylation of the same markers revealed by COBRA (Supplemental Table 1). This may be partially explained by a different location of CpG dinucleotides analysed by COBRA and Infinium DNA methylation assay (data not shown). It is likely that methylation of markers in epigenotypes that display less dense methylation across CpG islands (such as IME/LME) is more dependent on the exact location of analyzed CpGs (29). Therefore, all of the correlations here reported or negated need to be confirmed in other independent studies.

To determine whether relations between IME/LME and CIMP-low/CIMP-0 holds true also for well defined setting, we epigenotyped by COBRA six CRC cell lines that have been characterized previously according to their epigenotype status and/or CIMP status (Figure 2, Table 3) (10, 27). Our results were concordant with those described by Yagi et al., however, when we compared our epigenotyping to the CIMP status of the cell lines described by Hinoue et al. it became evident that all IME cell lines were defined as CIMP-0 (10, 27). This evaluation indicated that the lack of equivalency between IME/CIMP-low and LME/CIMP-0 is likely caused by a different set of markers used in CIMP/epigenotype determination rather than the pooling error or some other methodological issues. This could be supported by some differences

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between performance of Ogino's markers and Yagi's markers on classification of tumors to CIMP-low/CIMP-0 or IME/LME subgroups which has been previously shown by Yagi et al. (10). One possible cause is that Yagi et al. used cell lines to derive specific markers whereas markers decribed by Ogino et al. and Hinoue et al. were developed by screens of large groups of CRCs (3, 30). Moreover, one of the cell lines (SW480) used by Yagi et al. to identify markers to distinguish IME from LME exemplify aneuploid cell line exhibiting complex karyotype with many numerical and structural abnormalities (Table 3) (31-34). To provide evidence that use of SW480 as a marker "donor" for IME could favor tumors with copy number changes we performed CGH analysis of 100 previously epigenotyped CRCs (12). Indeed, IME tumors displayed apparently higher mean number of total chromosomal imbalances as well as significantly higher mean number of amplifications and deletions when compared to HME and LME (Table 2). Since a number of studies assigned indicators of chromosomal instability (such as LOH of selected loci and enrichment of *TP53* mutations) to CIMP-0 phenotype, our results provide a molecular link between IME and CIMP-0 and explain the reason of their similarities (8, 35, 36). Concerns, however, can be raised regarding significant enrichment of IME for *KRAS* mutations which have been regarded as an attribute of CIMP-low (7, 28). Interestingly, very recent findings suggest that *KRAS* mutation do not impose a unique methylation profile on CIMP-low and therefore *KRAS* mutation can not be regarded as cardinal classifier of CIMP-low/IME which is also underlined by the fact that *KRAS* mutations are present, to some extent, in CIMP-0/LME tumors (9, 12, 37).

In summary, our data show that IME tumors display features that have been assigned to CIMP-0 whereas LME tumors can be compared, to some extent, to CIMP-low phenotype except for lower *KRAS* mutations frequency. These

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discrepancies should be considered when interpreting the data from a particular epigenotyping method, however, more studies leading to validation of these findings are needed.

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Figure legends:

Figure 1. Genome-scale methylation profiling of HME, IME and LME DNA pools. (A) Principal components analysis of the methylation data of 3421 CpGs differentially methylated between CRC epigenotypes and normal colonic tissues. Each dot represent single DNA pool. (B) Schematic representation of set of 3421 CpGs in a Venn diagram showing the relationship of differentially methylated CpGs in the three epigenotypes.

Figure 2. Epigenotyping of CRC cell lines by combined bisulfite restriction assay (COBRA): *lane 1* - DLD1; *lane 2* - HTC116; *lane 3* - HT29; *lane 4* - CACO2; *lane 5* - SW480; *lane 6* - LOVO; *lane 7* - DNA pool combined from 50 normal colon tissues; *lane 8* – blank control (H_2O); lane 9 – GeneRuler Ultra Low Range DNA Ladder (Fermentas UAB, Vilnius, Lithuania). The level of methylation of markers for each lane is presented in the Supplemental Table 6.

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Tables

Table 1. Characteristics of DNA pools used in genome-wide methylation analysis

	HME *n* (%)	IME *n* (%)	LME *n* (%)	Normal *n* (%)
Number of samples	20	32	32	70
Number of pools	6	6	6	3
Age mean ± SD	66 ± 12	64 ± 12	63 ± 11	64 ± 11
Female	12 (60)	19 (60)	17 (53)	31 (44)
Male	8 (40)	13 (40)	15 (47)	39 (56)
Proximal	16 (80)	5 (16)	9 (28)	26 (37)
Distal	4 (20)	27 (84)	23 (72)	44 (63)
BRAF mutant	12 (60)	0 (0)	0 (0)	-
BRAF wild-type	8 (40)	32 (100)	32 (100)	-
KRAS mutant	0 (0)	14 (44)	12 (37)	-
KRAS wild-type	20 (100)	18 (56)	20 (63)	-
MSI	10 (50)	0 (0)	0 (0)	-
MSS	10 (50)	32 (100)	32 (100)	-

Table 2. Clinical and molecular characteristics of 100 CRCs selected for CGH and the mean of copy number changes estimated by CGH using HME, IME and LME CRC clusters

Clinical and molecular Characteristics	Total n (%)§	HME n (%)§	IME n (%)	LME n (%)	p-value (HME vs IME)	p-value (HME vs LME)	p-value (IME vs LME)	p-value (HME vs IME vs LME)
	100 (100)	20 (20)	40 (40)	40(40)				
Age mean ± SD	65 ± 11	67± 13	65 ± 10	65 ± 11	0.517	0.611	0.868	0.775
Female	48 (48)	13 (65)	18 (45)	17 (42)	0.177	0.170	1.000	0.271
Male	52 (52)	7 (35)	22 (55)	23 (58)				
Proximal	28(28)	15 (75)	8 (20)	5 (12)	5.88×10^{-5}	2.54×10^{-6}	0.546	2.95×10^{-6}
Distal	72 (72)	5 (15)	32 (80)	35 (88)				
BRAF mutant	15 (15)	14 (70)	0 (0)	1 (2)	5.88×10^{-9}	2.54×10^{-8}	1.000	2.95×10^{-11}
BRAF wild-type	85 (85)	6(30)	40 (100)	39 (98)				
KRAS mutant	30 (30)	2 (10)	21 (52)	11 (27)	0.003	0.180	0.04	0.00461
KRAS wild-type	70(70)	18 (90)	19 (48)	29 (73)				
MSI	14 (14)	11 (55)	2 (5)	1 (2)	2.64×10^{-5}	4.89×10^{-6}	1.000	3.22×10^{-7}
MSS	86 (86)	9 (45)	38 (95)	39 (98)				
CGH mean changes ± SD	7.6 ± 6.5	4.5 ± 5.5	10.4 ± 6.9	6.25 ± 5.6	0.001	0.142	0.004	0.001
CGH mean amplifications ± SD	3.7 ± 3.6	1.9 ± 2.8	4.7 ± 3.7	3.4 ± 3.6	0.001	0.045	0.064	0.003
CGH mean deletions ± SD	3.9 ± 4.0	2.5 ± 3.6	5.7 ± 4.5	2.8 ± 3.0	0.007	0.418	0.003	0.003

SD – standard deviation

§ - data adapted from Kozlowska J, Karpinski P, Szmida E, Laczmanska I, Misiak B, Ramsey D, Bebenek M, Kielan W, Pesz KA, Sasiadek MM. Assessment of chromosomal imbalances in CIMP-high and CIMP-low/CIMP-0 colorectal cancers. Tumour Biol. 2012;33:1015-9.

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Table 3. Epigenetic and cytogenetic status of six selected colorectal cancer cell lines

| Cell Line	Epigenotyping by COBRA (this study)	Epigenotyping by Yagi et al.[9]	Epigenotyping by Hinoue et al.[13]	Ploidy[§]	Numerical abnormalities (+/-)[§]	Translocations[§]	Copy number changes[§]
DLD1	HME	HME	CIMP-high	Diploid	0	1	3
HTC116	HME	HME	CIMP-high	Near-diploid	1	5	4
HT29	HME	N.D[†]	CIMP-high	Triploid	6	8	20
CaCo2	IME	IME	CIMP-0	Aneuploid	18	16	N.D[†]
SW480	IME	IME	CIMP-0	Hyperdiploid	6	15	18
LOVO	HME	HME	N.D[†]	Near-diploid	3	1	4

[§] References: Knutsen et al. (31); Kleivi et al. (32); Abdel-Rahman et al. (33) ; Ghadimi et al. (34)
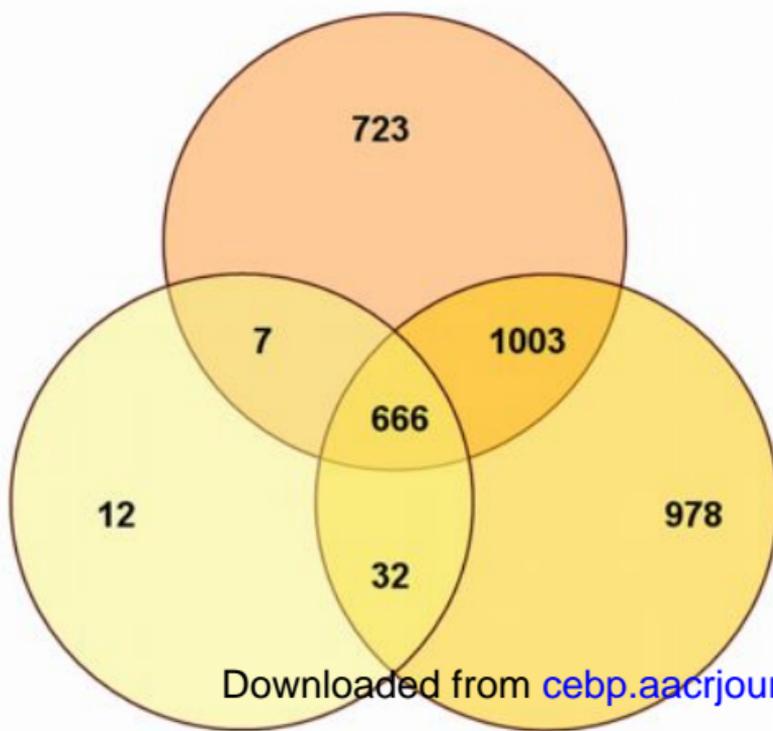
[†] N.D – not defined

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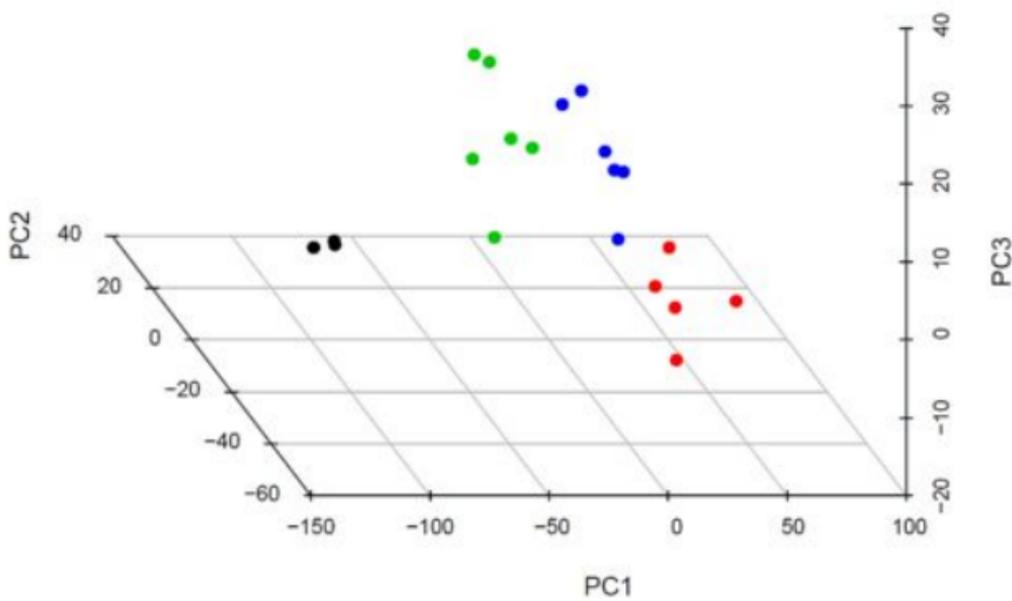
Figure 1

Figure 2

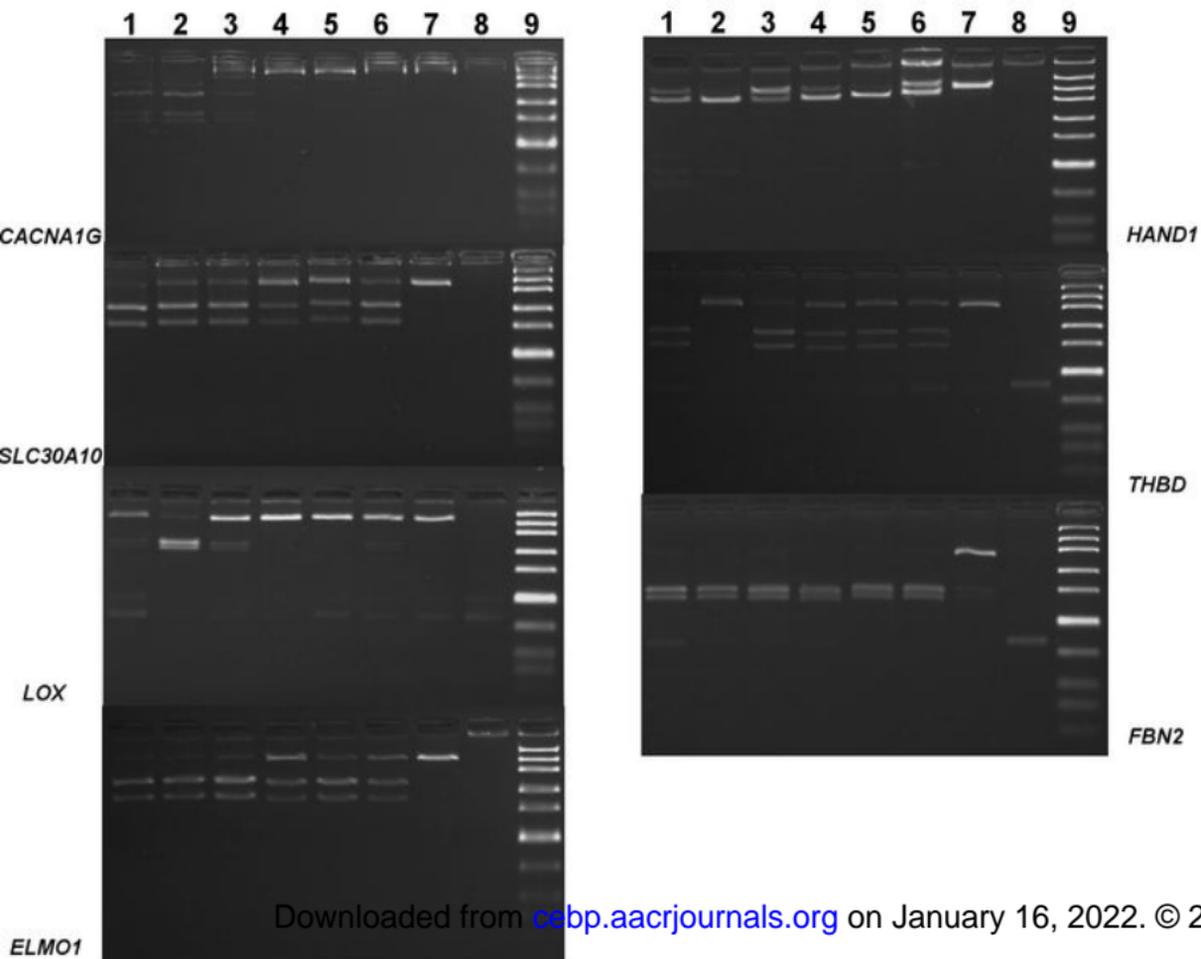# Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

Intermediate- and low-methylation epigenotypes do not correspond to CpG island methylator phenotype (low and -zero) in colorectal cancer

Pawel Karpinski, Michael Walter, Elzbieta Szmida, et al.

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