Abstract

Neoplastic growth is frequently associated with genomic DNA methylation that causes transcriptional silencing of tumor suppressor genes. We used a collection of colorectal polyps and carcinomas in combination with bioinformatics analysis of large datasets to study the expression and methylation of *Hypermethylated in cancer 1* (*HIC1*), a tumor suppressor gene inactivated in many neoplasms. In premalignant stages, *HIC1* expression was decreased, and the decrease was linked to methylation of a specific region in the *HIC1* locus. However, in carcinomas, the *HIC1* expression was variable and, in some specimens, comparable to healthy tissue. Importantly, high *HIC1* production distinguished a specific type of chemotherapy-responsive tumors.

Introduction

Cancer is viewed as a genetic disease caused by mutations of protooncogenes and tumor suppressors. It is presumed that the first oncogenic mutation provides selective advantage to the prospective cancer cells that multiply and form the initial neoplastic lesion. Moreover, solid tumors develop over time by acquiring additional alterations that drive tumor progression (reviewed in [1]). These alterations might include extensive changes in methylation of genomic DNA that (presumably) contribute to tumorigenesis by transcriptional silencing of tumor suppressor genes (reviewed in [2]). Adenocarcinomas affecting colon and rectum [colorectal carcinoma (CRC)] represent a major cause of morbidity and mortality in developed countries. Colorectal cancer is the third most common malignancy worldwide and the fourth most common cause of
cancer-related death [3]. Recently, several research consortia analyzed numerous CRCs using massive parallel sequencing and DNA microarray technologies in a comprehensive manner, yielding an unprecedented insight into the pathobiology of CRC. Consequently, the commonly accepted division of CRCs into microsatellite instability, neoplasm frequently associated with increased mutation rates and CpG island methylated phenotype (CIMP), and those that display microsatellite instability but are chromosomally unstable, was recently revised (reviewed in [4]). Based on the differences in global expression profiles, six independent classification systems were proposed, dividing CRCs into three to six distinct molecular subtypes [5–10]. Very recently, reevaluation of published large-scale data resulted in subdivision of CRCs into four consensus molecular subtypes (CMSs) [11]. Importantly, because RNA expression profiles are tightly linked to the tumor phenotype, CRC molecular classification not only might improve the patient staging system but also could identify molecules or pathways for specific therapeutic interventions.

HIC1 is a tumor suppressor gene located on chromosome 17p13.3, a chromosomal region that is frequently hypermethylated, rearranged, or lost in various human cancers, including colon, lung, brain, breast, prostate, and leukemia (reviewed in [12]). The HIC1 gene encodes an evolutionarily conserved transcriptional repressor. It is presumed that HIC1 promoter hypermethylation leads to its silencing; however, only a limited number of reports have shown the relation between HIC1 methylation and expression, or the results are conflicting. For example, HIC1 promoter hypermethylation was detected in healthy brain and prostate, but because the level of the HIC1 gene expression was not determined, no conclusion could be drawn from the observation [13–15]. Additionally, during liver carcinogenesis, the HIC1 locus was increasingly methylated compared with the healthy tissue, precancerous lesions, and primary hepatocellular carcinomas. However, HIC1 expression did not correlate significantly with the extent of genomic DNA methylation [16,17].

In the present study, we analyzed the HIC1 expression pattern and locus methylation during neoplastic progression of human colorectal tumors. We used a collection of polyps and carcinomas, and, in addition, we employed large publicly available datasets that included gene expression profiling, high-throughput sequencing, and DNA methylation data of more than 970 human CRC specimens. We show that in premalignant stages, HIC1 expression is indeed decreased, and the decrease is related to the extent of DNA methylation in a specific region in the HIC1 locus. However, in CRC, HIC1 expression is more variable, and some specimens display HIC1 mRNA levels comparable to those observed in healthy tissue. Importantly, high HIC1 production distinguishes stroma-rich tumors responsive to chemotherapy.

**Materials and Methods**

**Patients and Tissue Samples**

Paired samples of normal and neoplastic tissue were obtained from 74 patients undergoing either polypectomy or surgical resection of sporadic CRC (Supplementary Table S1). The central portion of the tumor and corresponding normal colonic mucosa from the resection margin or at least 10 cm from the site of polypectomy were taken, immediately frozen, and stored in liquid nitrogen. None of the patients underwent radiotherapy or chemotherapy before the operation. The study was approved by the Ethics Committee of the Third Faculty of Medicine, Charles University, in Prague, and all patients signed the written consent to participate in the study.

**Total RNA and Genomic DNA Isolation, cDNA Synthesis**

Deep-frozen tissue samples were disrupted in 600 μl of lysis buffer by ceramic beads during one run of MagNa Lyser Instrument (Roche Life Sciences), and total RNA and genomic DNA were extracted using AllPrep DNA/RNA Mini kit (Qiagen) according to manufacturer’s instructions. The cDNA synthesis was performed in 20-μl reaction using 1 μg of total RNA, random hexamers, and RevertAid reverse transcriptase (Thermo Scientific) according to the manufacturer’s protocol.

**Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)**

Reactions were run in triplicates using LightCycler 480 Probes Master and Universal ProbeLibrary hydrolysis probes and Light-Cycler 480 Instrument (Roche Life Sciences). The primer pairs and corresponding Universal ProbeLibrary probes are listed in Supplementary Table 2. Crossing point-PCR-cycle (Cp) values for each triplicate were normalized by geometric average of housekeeping genes *ubiquitin B (UBB)* and *TATA box binding protein* (TBP). Resulting values were averaged to obtain ΔCp values for biological replicates. Relative mRNA abundance (ΔCp in healthy tissue – ΔCp in neoplastic tissue) was correlated with the histological grade of tumor samples using the rank-order Spearman’s (ρ) and Kendall’s (τ) coefficient. Neoaplastic progression was assumed as follows: hyperplasia (Hyp) < low-grade dysplasia (LGD) < high-grade dysplasia (HGD) < CRC [18].

**HIC1 Immunohistochemistry**

Five-micrometer sections from formalin-fixed and paraffin-embedded tissue samples were dewaxed in xylene and rehydrated. To unmask the antigen sites, the sections were immersed in a steam bath (20 minutes) in Target Retrieval Solution (Dako). Primary rabbit anti-HIC1 polyclonal antibody (ab33029, Abcam) was applied at 1:200 dilution overnight at 4°C. The primary antibody was detected using EnVision+ System (Dako), and the brown color reaction was developed with DAB (Vector Laboratories) substrate.

**DNA Methylation Analysis of the HIC1 Locus in Patient Samples**

Genomic DNA was isolated from deep-frozen tissue (disintegrated by MagNa Lyser System; Roche Life Sciences) using AllPrep DNA/RNA Mini kit (Qiagen). Bisulfite modification of genomic DNA (0.5 μg) from healthy and neoplastic tissue from HGD (n = 5) and CRC (n = 6) patients was performed using Imprint DNA Modification Kit (Sigma-Aldrich). Bisulfite-treated DNA was amplified using KAPA 2G Robust Hot Start ReadyMix (KapaBiosystems) and the following primers: hFHIC1: 5′-ATTATTTTTTTTTAATGGGTTAAATTTTTTTT-3′ and hRHIC1: 5′-CCTGAGAACCTTTACCTCTACTAATCTACATTTTTT-3′. The purified products were subcloned into the pGEM-T-Easy vector (Promega), and at least eight positive clones were sequenced. Sequencing data for CpG methylation were analyzed using the online quantification tool for methylation analysis (QUMA) (Laboratory for Mammalian Epigenetic Studies, Center for Developmental Biology, RIKEN).

**Public CRC Datasets and Statistical Analysis**

The GSE33113 [6] dataset was retrieved from ArrayExpress [19] (accession E-GEOD-33113); the GSE13294 [20], GSE14333 [21], and GSE62080 [22] datasets were retrieved from the Gene
Expression Omnibus [23] repository. The Cancer Genome Atlas (TCGA) data were accessed from the TCGA repository (Broad Institute, Massachusetts Institute of Technology, USA) on April 13, 2015. The expression, methylation, and mutational analyses are described in Supplementary material. All computations and statistical tests were performed in the R environment [24].

Results and Discussion

**Region-Specific Methylation of the HIC1 locus in CRC**

To determine changes in the HIC1 expression levels during neoplastic progression, we analyzed total RNA isolated from the matched healthy colonic mucosa and neoplastic tissue obtained from 74 patients. In adenomas, we observed significant decreases in HIC1 expression in polyps with low- and high-grade dysplasia when compared with healthy or hyperplastic tissue. HIC1 expression in CRCs was more heterogeneous, with median positioned between healthy/hyperplastic and dysplastic samples (Figure 1A). To explore the relationship between HIC1 expression and the methylation status of CRCs, we interrogated the relevant dataset recently available from the TCGA consortium (https://tcga-data.nci.nih.gov/tcga/; 277 specimens in total; the dataset was designated TCGA 450K; see Supplementary Materials and Methods for details). Unsupervised clustering of DNA methylation profiles identified four subgroups of tumors. Two of the subgroups contained CRCs with elevated methylation and were classified according to previously published nomenclature [25,26] as CIMP-high and CIMP-low. The two non-CIMP clusters were designated non–CIMP I and non–CIMP II. In addition, we employed TCGA gene expression data and determined (relative) HIC1 mRNA expression levels in each CRC. Samples that (according to HIC1 expression) fell into the first and last decile were defined as “HIC1-high” and “HIC1-low,” respectively. The majority of the HIC1-high samples were distributed among the CIMP-low, non–CIMP I, and non–CIMP II subgroups. Surprisingly, we did not observe clear correlation between the CRC CIMP status and HIC1 expression. In fact, one HIC1-high sample was found in the CIMP-high subgroup. Moreover, the HIC1-low samples were “scattered” among all four subgroups (Figure 1B). To analyze the methylation rate of HIC1, we evaluated the methylation status of the HIC1 locus. A substantial portion of mammalian genes contain the so-called CpG island, a promoter region rich in CpG nucleotides that are frequently methylated (reviewed in [27]). However, several studies have shown that most of the methylation changes in cancer DNA do not occur in the promoter CpG islands but in the regions located up to 2 kbp apart or even more distant. Importantly, the methylation status of these CpG island “shores” or “shelves” is strongly related to gene expression [28–30]. Up to six different HIC1 isoforms were detected in various tissues with predominance of HIC1α mRNA. These isoforms are produced by differential splicing or usage of three alternative promoters (reviewed in [31]). Accordingly, two CpG islands and adjacent shores and shelves (including all three promoters) were assigned to the locus (Figure 2A).

Using the TCGA 450K dataset, we retrieved information about methylation of 62 DNA probes covering a large portion of the HIC1 gene. Methylation probes with median of the methylated fraction equal to or greater than 50% (beta value ≥ 0.5) in the CIMP-high subgroup and median of the methylated fraction equal to or lesser than 20% (beta value ≤ 0.2) in both non-CIMP subgroups were considered as CIMP status-related (Supplementary Figure S1). Additionally, methylation probes whose methylation correlated with decreasing HIC1 expression (LM-fit coefficient ≤ -1) were identified as expression-related (Supplementary Figure S2). Interestingly, we identified 31 CIMP-related methylation probes that were located exclusively in CpG island 1. In contrast, all seven probes related to HIC1 expression were found in CpG island/shore 2. Subsequently, we confirmed the observation by testing methylation of genomic DNA isolated from our collection of healthy and neoplastic colorectal tissue. The cytosine methylation analysis was performed in the shore region upstream to CpG island 2 because the very high CG content of the island itself precluded any unbiased evaluation (Figure 3A). Analysis of the sequence containing 10 CpG

Figure 1. Analysis of HIC1 expression in colorectal neoplasia. (A) HIC1 expression changes during tumor progression. qRT-PCR analysis of the HIC1 mRNA levels in healthy tissue, hyperplastic adenomas (Hyp; n = 9), adenomas displaying low-grade (LGD; n = 24) or high-grade (HGD; n = 25) dysplasia, and CRC (n = 12). The boxed areas correspond to the second and third quartiles; the median of ΔCp values for each category is indicated as the red line. The relation between the HIC1 expression profile and neoplasia progression is significant, as evidenced by the Spearman’s (ρ = −0.67) and Kendall’s (τ = −0.51) coefficient values. (B) HIC1 expression in CRC subgroups clustered according to the DNA methylation profiles. HIC1-high and HIC1-low samples are indicated by brown or blue dots, respectively. Black dots indicate the other specimens. The red lines correspond to the median values; log2-expression intensity, binary logarithm of expression intensity (additional details are given in Supplementary Methods).
doublets located in (or surrounding) the second HIC1 exon revealed increased methylation in DNA isolated from HGD specimens when compared with DNA obtained from the matched healthy mucosa (Figure 3B). Additionally, in CRCs, we observed a clear difference in the methylation status between the HIC1-high and HIC1-low specimens (Figure 3C). We noted a complete absence of methylation in the expression-related region in one sample from the HIC1-low group (red arrow in Figure 2). However, subsequent copy-number variation analysis revealed deletion of the chromosomal region harboring the HIC1 locus in this particular sample, thus explaining the lack of the signal. HIC1 transcription is activated by p53 [32,33], and we therefore analyzed TP53 gene mutations in TCGA 450K CRCs. However, we did not find any significant differences in the TP53 status in HIC1-high [3 specimens harboring wild-type (wt) and 21 specimens mutant TP53] when compared with HIC1-low samples (5 wt vs 15 mutant specimens). Nevertheless, this does not

Figure 2. Identification of the CIMP-related and gene expression–related HIC1 methylation sites. Top, the HIC1 gene exon-intron structure indicating the position of the major HIC1a transcript driven from the P1 promoter; the position of two alternative promoters P2 and P0 is also shown. A color-based classification of two CpG islands and other elements is indicated under the schemes; methylation probes covering the HIC1 locus are indicated as horizontal bars. The bottom panels depict the HIC1 methylation status in 277 CRC cases present in the TCGA 450K dataset. In the left panel, the specimens were grouped according to the CIMP status of tumor DNA; each specimen is represented by a horizontal bar. The right panel shows the HIC1 methylation status in CRCs classified as HIC1-high or HIC1-low; the sample with the HIC1 locus deletion is indicated by red arrow. The extent of methylation of CIMP-related (yellow) and expression-related probes (green) is shown as the gray-shaded heatmap of beta values (unmethylated: white; completely methylated: black); notice the different “slope” of beta values between the left and right panel. Probes are listed vertically with their identification code in a successive order according to their target sequence coordinates (the 5′ gene end is shown on the left). The CRC methylation profile of four probes boxed in pink is shown in Supplementary Figure S1. The correlation between HIC1 expression and the methylation profile of these four probes is given in Supplementary Figure S2.
exclude that a transcriptional mechanism other than p53-dependent might—next to the epigenetic modifications—contribute to HIC1 expression.

**High HIC1 Expression in CRCs Responsive to Chemotherapy**

Next, we asked whether the HIC1 expression level is connected with some specific pathological CRC features using information contained in the TCGA 450K and additional three datasets TCGA 27K [34], GSE13294 [20], and GSE14333 [21] (the latter two datasets were processed together). Rather surprisingly, that is, considering the tumor suppressive role of HIC1, patients with HIC1-high CRCs displayed lower survival than patients with HIC1-low CRCs, although a significant difference was reached in the TCGA 27K dataset only (Figure 4A). Interestingly, an opposite phenomenon, that is, association of higher HIC1 expression with better clinical outcome, was observed in breast [35] or lung cancer [36]. The discrepancy might be attributed to the fact that more progressed malignancies prevailed among HIC1-high CRCs (Supplementary Figure S3). On the other hand, according to cancer-treatment related information retrieved from the GSE13294, GSE14333, and GSE62080 [22] datasets, patients from the HIC1-high group responded significantly better to FOLFIRI treatment than...
patients from the HIC1-low group (Figure 4B and Supplementary Figure S4). Of note, FOLFIRI, that is, combination of folinic acid (FOL/leucovorin), 5-fluorouracil, and topoisomerase inhibitor irinotecan (IRI), is used as the first-line therapy to improve maximal survival of patients with metastatic CRCs[37]. Based on expression profiles, we clustered CRCs from these datasets to stem-like, enterocyte, transit-amplifying, goblet-like, and inflammatory subtypes[9]. Interestingly, 53% of HIC1-high tumors (n=21) were assigned to the stem-like tumors that were associated with clinical benefits of FOLFIRI treatment (Figure 4B).

The HIC1-High CRC Group and CMS4 Cancer Subtype Relationship

Finally, we obtained another CRC dataset, GSE33113[6], and employed expression data representing 971 CRCs in total to identify the expression signature that distinguishes HIC1-high from HIC1-low CRC samples. Unbiased hierarchical clustering revealed 298 genes whose expression differed significantly between HIC1-high and HIC1-low tumors (Figure 5A, Supplementary Table S3). All these genes except C2 calcium-dependent domain containing 4A (C2CD4A) were upregulated in HIC1-high samples. This indicated—considering the transcriptionally repressive function of HIC1—that the selected genes are not directly regulated by HIC1. Indeed, none of the previously identified HIC1-repressed genes[38–48] was present among the genes. Nevertheless, qRT-PCR analysis of C2CD4A and five other genes highly upregulated in HIC1-high samples using eight CRC samples from our experimental collection (four CRCs were selected as HIC1-high, additional four as HIC1-low) supported the observation made “in silico” (Figure 5B).

Additionally, the obtained gene set was evaluated using the Enricher gene library online tool (http://amp.pharm.mssm.edu/Enrichr/) [49]. We performed gene set enrichment analysis to assign the “Gene Ontology Biological Processes” (GO Biological Processes), Kyoto Encyclopedia of Genes and Genomes pathways, and “GO Cellular Components” categories to the signature genes. The analysis revealed that the majority of identified genes encode secreted proteins that are involved in extracellular matrix organization and focal adhesion (Supplementary Figure S5, Supplementary Table S4).

Subsequently, we stratified individual CRC samples into four recently defined CMSs[11]. Strikingly, all HIC1-high samples were present in the CMS4 subtype (Figure 5C) that is characterized by stromal invasion, activation of transforming growth factor β (TGF-β) signaling, and extracellular matrix remodeling and complement-mediated inflammatory pathways. Indeed, genes encoding the TGF-β1 ligand and complement components were present among upregulated genes in HIC1-high tumors (Supplementary Table S4). Very recently, Calon and colleagues described an aggressive stem-like/mesenchymal CRC subtype with poor prognosis. Strikingly, the expression profile of this particular CRC subtype is related to the gene expression program induced by TGF-β in tumor stromal cells[50]. To this end, we compared genes upregulated in HIC1-high tumors with the TGF-β response gene signatures in different types of stromal cells. We found significant overlaps of gene profiles by TGF-β–activated normal colonic fibroblasts (including HIC1 that was among the TGF-β–activated genes), macrophages, and endothelial cells (Supplementary Table S5). This would suggest that (in some CRCs) HIC1 is expressed in stromal rather than in epithelial tumor cells. However, immunohistochemical staining clearly revealed HIC1-positive nuclei (and the cytoplasm) in the healthy colonic epithelium and in tumor cells in HIC1-high CRC samples. Additionally, HIC1 positivity was detected in the nuclei of cells present in the lamina propria and in tumor stroma of both HIC1-high and HIC1-low samples (Figure 5D).

Figure 4. HIC1-high CRCs are responsive to FOLFIRI treatment. (A) Kaplan-Meier plots of patient survival for indicated datasets. (B) Assignment of HIC1-high/low CRCs from the GSE13294 and GSE14333 datasets according to CRC subtype clustering and FOLFIRI response as described in reference[9]. *P < .05; ***P < .001 (Student’s t test).
Cancer-associated fibroblasts are the main source of TGF-β ligand in colorectal tumors [51]. Interestingly, Chen and colleagues reported that the HIC1 gene was induced in primary lung cancer cells cocultured with cancer-associated fibroblasts [52]. In addition, exposure of non–small cell lung cancer cells to TGF-β also led to the increased HIC1 mRNA levels [53]. Of note, both observations were obtained upon interrogation of the corresponding datasets deposited by the authors to the ArrayExpress repository at https://www.ebi.ac.uk/arrayexpress. Finally, Briones and colleagues found that the repression of fibroblast growth factor-binding protein 1 by TGF-β signaling depends on HIC1 [40]. These data suggest a functional relationship between cancer and stromal (i.e., TGF-β-producing) cells in HIC1-expressing CRCs. In contrast, Calon and colleagues reported that because of the mutations in genes encoding components of TGF-β signaling, many epithelial cancer cells are not responsive to the TGF-β cues [50]. To solve this possible discrepancy, we used exome sequence data from the TCGA 450K dataset to identify mutations in the TGFβ1 branch of TGF-β signaling in the CMS4 group of tumors. We found frequent missense mutations in the SMAD4 gene encoding the common cytoplasmic/nuclear mediator of the signaling. However, these mutations were equally distributed among HIC1-high and “other” tumors. Nonetheless, none of the identified inactivating mutations [affecting genes encoding TGFBR1, SMAD2, and SMAD4] was found in HIC1-high samples. Interestingly, in 17 of 50 CRCs, we did not detect any mutations in the TGFβ1-dependent branch of the pathway (Supplementary Figure S6). This might imply that epithelial cancer cells in (some) stroma-rich CRCs retained TGF-β responsiveness. How is HIC1 (re)expression in CRC regulated? What is the function of the protein in progressed CRC, and why is a portion of the HIC1 protein in tumor cells cytoplasmic? These intriguing questions remain to be elucidated. However, our results clearly indicate that HIC1 expression defines a group of CRCs sensitive to FOLFIRI treatment. The study also documents how high-throughput approaches started...
to shape current biomedical and molecular biology research. Clinically relevant data can be produced by “data mining” of large publicly available datasets that are complemented by experiments using a relatively small collection of clinical samples.

Conclusions
(1) Region-specific methylation dictates the HIC1 expression levels in intestinal cancer.
(2) High HIC1 production distinguishes stroma-rich carcinomas responsive to chemotherapy.

Conflict of Interest
There are no conflicts of interest to disclose.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.tranon.2016.01.005.

References
[1] Kinzler KW and Vogelstein B (1996). Lessons from hereditary colorectal cancer. Cell 87, 159–170.
[2] Jones PA and Baylin SB (2002). The fundamental role of epigenetic events in cancer. Nat Rev Genet 3, 415–428.
[3] Siegel R, Desantis C, and Jemal A (2014). Colorectal cancer statistics, 2014. CA Cancer J Clin 64, 104–117.
[4] Van Schaeybroeck S, Allen WL, Turkington RC, and Johnston PG (2011). HIC1 (hypermethylated in cancer 1) epigenetic silencing in tumors. BMC Med Genomics 4, 64.
[5] Budinska E, et al (2013). Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer. J Pathol 231, 63–76.
[6] De Sousa EMF, et al (2013). Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. Nat Med 19, 614–618.
[7] Matisa L, et al (2013). Gene expression classification of colon cancer into molecular subtypes: characterization, validation, and prognostic value. PLoS Med 10(6), e1001453.
[8] Roepman P, et al (2014). Colorectal cancer intrinsic subtypes predict chemotherapy benefit, deficient mismatch repair and epithelial-to-mesenchymal transition. Int J Cancer 134, 552–562.
[9] Sadanandam A, et al (2013). A colorectal cancer classification system that associates cellular phenotype and responses to therapy. Nat Med 19, 619–625.
[10] Schlicker A, et al (2012). Subtypes of primary colorectal tumors correlate with response to targeted treatment in colorectal cell lines. BMC Med Genomics 5, 66.
[11] Guiney J, et al (2015). The consensus molecular subtypes of colorectal cancer. Nat Med 21, 1350–1356.
[12] Fleurid C, Touka M, Boulay G, Guerardel C, Rood BR, and Leprince D (2009). HIC1 (hypermethylated in cancer 1) epigenetic silencing in tumors. Int J Biochem Cell Biol 41, 26–33.
[13] Morton Jr RA, Watkins JJ, Bova GS, Baylin SB, and Isaacs WB (1996). Hypermethylation of chromosome 17P locus D17S5 in human prostate tissue. J Urol 156, 512–516.
[14] Rood BR, Zhang H, Weitman DM, and Cogen PH (2002). Hypermethylation of HIC-1 and 17p allelic loss in medulloblastoma. Cancer Res 62, 3794–3797.
[15] Uhlmann K, et al (2003). Distinct methylation profiles of glioma subtypes. Int J Cancer 106, 52–59.
[16] Kazai Y, Hui AM, Sun L, Ushijima S, Sakamoto M, Tsuda H, and Hirohashi S (1999). DNA hypermethylation at the D17S5 locus and reduced HIC-1 mRNA expression are associated with hepatocarcinogenesis. Hepatology 29, 763–709.
[17] Nishida N, Nagasaka T, Ninimura T, Ikai I, Boland CR, and Goel A (2008). Aberrant methylation of multiple tumor suppressor genes in aging liver, chronic hepatitis, and hepatocellular carcinoma. Hepatology 47, 908–918.
[18] Schlemper RJ, et al (2000). The Vienna classification of gastrointestinal epithelial neoplasia. Gut 47, 251–255.
[19] Kolesnikov N, et al (2015). ArrayExpress update—simplifying data submissions. Nucleic Acids Res 43, D1113–D1116.
[20] Jorissen RN, et al (2008). DNA copy-number alterations underlie gene expression differences between microsatellite stable and unstable colorectal cancers. Clin Cancer Res 14, 8061–8069.
[21] Jorissen RN, et al (2009). Metastasis-associated gene expression changes predict poor outcomes in patients with Dukes Stage B and C colorectal cancer. Clin Cancer Res 15, 7642–7651.
[22] Del Rio M, et al (2007). Gene expression signature in advanced colorectal cancer patients select drugs and response for the use of leucovorin, fluorouracil, and irinotecan. J Clin Oncol 25, 773–780.
[23] Barret T, et al (2013). NCBI GEO: archive for functional genomics data sets—update. Nucleic Acids Res 41, D991–D995.
[24] Team RC (2014). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing-3–900051-07-0; 2014.
[25] Hinoue T, et al (2012). Genome-scale analysis of aberrant DNA methylation in colorectal cancer. Genome Res 22, 271–282.
[26] Kawasaki T, Ohnishi M, Nosho K, Suemoto Y, Kinkg JG, Meyerhardt JA, Fuchs CS, and Ogino S (2008). CpG island methylation phenotyp-low (CIMP-low) colorectal cancer shows not only few methylated CIMP-high-specific CpG islands, but also low-level methylation at individual loci. Mod Pathol 21, 245–255.
[27] Jones PA (2012). Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 13, 484–492.
[28] Berman BP, et al (2012). Regions of focal DNA hypermethylation and long-range hypomethylation in colorectal cancer coincide with nuclear lamina-associated domains. Nat Genet 44, 40–46.
[29] Bibikov M, et al (2011). High density DNA methylation array with single CpG site resolution. Genomics 98, 288–295.
[30] Irizarry RA, et al (2009). The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. Nat Genet 41, 178–186.
[31] Jenal M, Britschgi C, Fey MF, and Tschan MP (2010). Inactivation of the hypermethylated in cancer 1 tumour suppressor—not just a question of promoter hypermethylation! Swiss Med Wkly 140, w13106.
[32] Mondal AL, Chinnadurai S, Datta K, Chauhan SS, Sinha S, and Chattopadhyay P (2006). Identification and functional characterization of a novel unspecific transcript variant of HIC-1 in human cancer cells exposed to adverse growth conditions. Cancer Res 66, 10466–10477.
[33] Wales MM, Beld MA, el Deiry W, Nelkin BD, Issa JP, Cavenek WK, Kuebriiz SJ, and Baylin SB (1995). p53 activates expression of HIC-1, a new candidate tumour suppressor gene on 17p13.3. Nat Med 1, 570–577.
[34] Cancer Genome Atlas Research, N., et al (2013). The Cancer Genome Atlas Pan-Cancer analysis project. Nat Genet 45, 1113–1120.
[35] Nicol G, Crichton DN, McDowell HE, Kernohan N, Hupp TR, and Thompson AM (2001). Expression of the hypermethylated in cancer gene (HIC-1) is associated with good outcome in human breast cancer. Br J Cancer 85, 1878–1882.
[36] Hayashi M, et al (2001). Reduced HIC-1 gene expression in non-small cell lung cancer and its clinical significance. Anticancer Res 21, 535–540.
[37] Grothey A, Sargent D, Goldberg RM, and Schmoll HJ (2004). Survival of patients with advanced colorectal cancer improves with the availability of fluourouracil-leucovorin, irinotecan, and oxaplatin in the course of treatment. J Clin Oncol 22, 1209–1214.
[38] Boulay G, Malauquin N, Loison I, Foveau B, Van Rechem C, Rood BR, Pourtier A, and Leprince D (2012). Loss of hypermethylated in cancer 1 (HIC1) in breast cancer cells contributes to stress-induced migration and invasion through beta-2 adrenergic receptor (ADRB2) misregulation. J Biol Chem 287, 5379–5389.
[39] Briggs KJ, Corcoran-Schwartz IM, Zhang W, Hacette T, Devereux WL, Baylin SB, Eberhart CG, and Watkins DN (2008). Cooperation between the Hic1 and Pch1 tumor suppressors in medulloblastoma. Genes Dev 22, 770–785.

[40] Briones VR, Chen S, Riegel AT, and Lechleider RJ (2006). Mechanism of fibroblast growth factor-binding protein 1 repression by TGF-beta. Biochem Biophys Res Commun 345, 595–601.

[41] Dehennaut V, Loison I, Boulay G, Van Rechem C, and Leprince D (2013). Identification of p21 (CIP1/WAF1) as a direct target gene of HIC1 (hypermethylated in cancer 1). Biochem Biophys Res Commun 430, 49–53.

[42] Foveau B, Boulay G, Pinte S, Van Rechem C, Rood BR, and Leprince D (2012). The receptor tyrosine kinase EphA2 is a direct target gene of hypermethylated in cancer 1 (HIC1). J Biol Chem 287, 5366–5378.

[43] Chen WY, Wang DH, Yen RC, Luo J, Gu W, and Baylin SB (2005). Tumor suppressor HIC1 directly regulates SIRT1 to modulate p53-dependent DNA-damage responses. Cell 123, 437–448.

[44] Janeckova L, et al (2015). HIC1 tumor suppressor loss potentiates TLR2/NF-kappaB signaling and promotes tissue damage-associated tumorigenesis. Mol Cancer Res 13, 1139–1148.

[45] Van Rechem C, Boulay G, Pinte S, Stankovic-Valentin N, Guerardel C, and Leprince D (2010). Differential regulation of HIC1 target genes by CtBP and NuRD, via an acetylation/SUMOylation switch, in quiescent versus proliferating cells. Mol Cell Biol 30, 4045–4059.

[46] Van Rechem C, et al (2009). Scavenger chemokine (CXC motif) receptor 7 (CXCR7) is a direct target gene of HIC1 (hypermethylated in cancer 1). J Biol Chem 284, 20927–20935.

[47] Vilgelm AE, Hong SM, Washington MK, Wei J, Chen H, El-Rifai W, and Zaika A (2010). Characterization of DeltaNp73 expression and regulation in gastric and esophageal tumors. Oncogene 29, 5861–5868.

[48] Zhang W, et al (2010). A potential tumor suppressor role for Hic1 in breast cancer through transcriptional repression of ephrin-A1. Oncogene 29, 2467–2476.

[49] Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR, and Ma’ayan A (2013). Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics 14, 128.

[50] Calon A, et al (2015). Stromal gene expression defines poor-prognosis subtypes in colorectal cancer. Nat Genet 47, 320–329.

[51] Calon A, et al (2012). Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. Cancer Cell 22, 571–584.

[52] Chen WJ, et al (2014). Cancer-associated fibroblasts regulate the plasticity of lung cancer stemness via paracrine signalling. Nat Commun 5, 3472.

[53] Sun Y, et al (2014). Metabolic and transcriptional profiling reveals pyruvate dehydrogenase kinase 4 as a mediator of epithelial-mesenchymal transition and drug resistance in tumor cells. Cancer Metab 2, 20.