**Imaging, Diagnosis, Prognosis**

**Jumonji Domain Containing 1A Is a Novel Prognostic Marker for Colorectal Cancer: *In vivo* Identification from Hypoxic Tumor Cells**

Mamoru Uemura¹, Hirofumi Yamamoto¹, Ichiro Takemasa¹, Koshi Mimori³, Hideyuki Hemmi¹, Tsunekazu Mizushima¹, Masataka Ikeda¹, Mitsugu Sekimoto³, Nariaki Matsuura², Yuichiro Doki¹, and Masaki Mori¹

**Abstract**

**Purpose:** This study aimed to identify novel hypoxia-inducible and prognostic markers *in vivo* from hypoxic tumor cells.

**Experimental Design:** Using carbonic anhydrase 9 and CD34 as a guide for hypoxic tumor cells, laser capture microdissection was used to isolate colorectal cancer (CRC) liver metastases. The samples were analyzed by microarray analysis, in parallel with five CRC cell lines cultured under hypoxic conditions. To evaluate the prognostic impact of the expression of certain genes, samples from a total of 356 CRC patients were analyzed by microarray or quantitative reverse transcription-PCR. *In vitro* mechanistic studies and *in vivo* therapeutic experiments were also done about a histone H3 Lys⁹ demethylase, Jumonji domain containing 1A (JMJD1A).

**Results:** Several candidate genes were identified by microarray analysis of liver metastases and culturing of CRC cells under hypoxic conditions. Among them, we found that JMJD1A was a novel independent prognostic factor for CRC (*P* = 0.013). *In vitro* assays revealed that loss of JMJD1A by small interfering RNA treatment was associated with a reduction of proliferative activity and decrease in invasion of CRC cell lines. Furthermore, treatment with an adenovirus system for antisense JMJD1A construct displayed prominent therapeutic effects when injected into established tumor xenografts of the CRC cell lines HCT116 and DLD1.

**Conclusions:** JMJD1A is a useful biomarker for hypoxic tumor cells and a prognostic marker that could be a promising therapeutic target against CRC.

**Hypoxia is a characteristic of many solid tumors. Intratumoral hypoxia affects every major aspect of cancer biology, including cell invasion, metastasis, and determination of cell death (1). Intratumoral hypoxia and/or expression of the hypoxia-related endogenous proteins, vascular endothelial growth factor (VEGF), carbonic anhydrase 9 (CA9), hypoxia-inducible factor-1 (HIF-1), and glucose transporter 1 (GLUT1), are predictive of a poor prognosis in breast cancer (2), head and neck tumors (3), non–small cell lung cancer (4), cervical cancer (5), and colorectal cancer (CRC; ref. 6). There is also evidence that a hypoxia-related gene expression profile is associated with poor prognosis in human cancers (7). Taken together, these findings indicate that hypoxic conditions contribute to aggressive tumor behavior and to a more malignant phenotype.**

Many molecules in the hypoxia-response pathway are good candidates for therapeutic targeting (8–10). The anti-VEGF antibody bevacizumab is used clinically for treatment of several human cancers (11), supporting that hypoxia-induced genes are clinically relevant therapeutic targets. Therefore, the identification of novel hypoxia-inducible genes holds great potential for the development of additional cancer therapies.

We aimed to identify, using microarray analysis, a novel prognostic factor and potential therapeutic target *in vivo* using hypoxic tumor cells from hepatic metastases of CRC. We found that Jumonji domain containing 1A (JMJD1A), a histone H3 Lys⁹ demethylase, was a useful biomarker for hypoxic tumor cells and a poor prognosis of CRC. JMJD1A functions as a modulator of transcriptional activation of downstream target genes (12–14).

**Authors’ Affiliations:** ¹Department of Surgery, Gastroenterological Surgery, Graduate School of Medicine and ²Department of Pathology, School of Allied Health Science, Faculty of Medicine, Osaka University, Osaka, Japan; and ³Department of Surgery and Molecular Oncology, Medical Institute of Bioregulation, Kyushu University, Beppu, Japan

**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

M. Uemura and H. Yamamoto contributed equally to this work.

**Corresponding Author:** Hirofumi Yamamoto, Department of Surgery, Gastroenterological Surgery, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita City, Osaka, 565-0871, Japan. Phone: 81-6-6879-3251; Fax: 81-6-6879-3259; E-mail: hyamamoto@gesurg.med.osaka-u.ac.jp.

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Translational Relevance

Hypoxia is a characteristic feature of many solid tumors. Intratumoral hypoxia affects every major aspect of cancer biology. However, it is not easy to detect truly important hypoxia-inducible genes that are related to clinical cancer biology in vivo because cancer cells usually exist in chronically hypoxic conditions in vivo with complex interactions with several pathways. In this study, we showed that liver metastasis of colorectal cancer is a useful in vivo material to identify novel hypoxia-inducible and prognostic markers. This finding has great potential for extending our knowledge of hypoxia-related cancer biology and may provide guidance for developing novel cancer therapies. Furthermore, we have shown that a histone H3 Lys3 demethylase, Jumonji domain containing 1A (JMJD1A), is a useful prognostic marker that can be a therapeutic target in colorectal cancer using the therapeutic xenograft model.

Because hypoxic tumor cells are likely to be resistant to cancer therapy, the present findings may provide clues for the development of a novel anticancer therapy.

Materials and Methods

Cell culture

HEK293 cells and human colon cancer cell lines HCT116, LoVo, DLD1, and HT29 were obtained from the American Type Culture Collection. The human colon cancer cell line KM12SM was a kind gift from Prof. T. Minamoto (Cancer Research Institute, Kanazawa, Japan). Cells were grown in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified incubator with 5% CO2. For culture under hypoxic conditions, cells were grown for up to 72 hours at 37°C in a humidified incubator with 5% CO2. For culture under hypoxic conditions, cells were grown for up to 72 hours at 37°C in a humidified incubator with 5% CO2.

Clinical samples

Hepatic metastases from CRC patients (n = 15) were consecutively collected exclusively during partial liver resection at Osaka University Hospital between 2000 and 2005. The samples of liver metastasis were embedded in OCT compound and frozen in liquid nitrogen within 10 to 15 minutes of resection. The samples were stored at −80°C until RNA extraction. For microarray analysis, we prospectively collected 214 primary CRC samples from consecutive patients who had curative operations in 2003 to 2006 from Osaka University Hospital and its nine associated hospitals. For quantitative reverse transcription-PCR (qRT-PCR), tumor samples were consecutively collected from a total of 142 CRC patients who had curative surgery from 1998 to 2002 at the Department of Surgery, Medical Institute of Bioregulation, Kyusyu University, and at its three associated institutes. The mean follow-up times were 44.0 ± 14.4 and 43.7 ± 33.5 months, respectively. The clinicopathologic features of patients from each institute, including gender, tumor location, extent of wall invasion, lymph node metastasis, histologic grade, clinical stage, and invasion to vein or lymphatic duct, are shown in Supplementary Table S1A and B.

In this study, none of the patients had preoperative chemotherapy or irradiation. After surgery, patients with stage III/IV tumors were basically treated with 5-fluorouracil–based chemotherapy. The surgical specimens were preserved in paraffin block and used for immunostaining of CD34 and JMJD1A. A piece of each primary CRC tissue sample was collected from the fresh specimens within 30 minutes after resection and stored in RNA Stabilization Reagent (RNALater, Ambion, Inc.) at −80°C until RNA extraction. The RNA samples were kept at −80°C. For long-time storage, cDNA was routinely synthesized from RNA samples within 4 to 6 weeks after the operation and stored at −20°C. The Human Ethics Review Committee of Osaka University and Kyusyu University approved the use of the resected samples. RE-MARK criteria for tumor marker studies was used for the preparation of this article (15).

Immunohistochemistry and vessel count

Immunohistochemical analysis was done as described previously (16). Frozen sections (8 μm) were fixed in 4% paraformaldehyde for 5 minutes. The anti-CD34 mouse monoclonal antibody (1:500; Novoceastra), anti-human CA9 goat polyclonal antibody (1:200; Santa Cruz Biotechnology), and anti-JMJD1A rabbit polyclonal antibody (1:100; Proteintech Group, Inc.) were incubated on the slides for 1 hour at room temperature. For negative controls, nonimmunized immunoglobulin G (Vector Laboratories) was used as a substitute for the primary antibody. Double staining of CD34 and CA9 was carried out as described previously (17). CA9 expression was scored as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. CD34+ blood vessels were counted under a microscope at ×100 magnification. Ten visual fields were selected randomly in each portion of the metastatic CRC lesion, and the mean vessel counts per visual field were calculated. Vessel count was scored as follows: 0, no vessel staining; 1, 1 to 3 vessels; 2, 4 to 10 vessels; and 3, >10 vessels.

Western blot analysis

Western blot analysis was done as described previously (18). The membrane was incubated with the primary antibodies at the appropriate concentrations (1:800 for JMJD1A and 1:1,000 for actin) for 1 hour.
Laser capture microdissection and microarray analysis

Laser capture microdissection (LCM) was done using the LM200 LCM system (Arcturus Engineering) as described previously (17). The quality check of total RNA and microarray analysis were carried out as described previously (19) using an oligonucleotide microarray covering 30,000 human probes (AceGene; DNA Chip Research, Inc. and Hitachi Software Engineering Co. Ltd.).

qRT-PCR

After reverse transcription, real-time monitoring of PCRs was done using the LightCycler system (Roche Applied Science) for quantification of mRNA expression (18). A housekeeping gene, porphobilinogen deaminase (PBGD), was used as an internal control (20). The PCR primers used in this study are listed in Supplementary Table S2.

Transfection of small interfering RNA

For small interfering RNA (siRNA) inhibition, double-stranded RNA duplexes targeting human JMJD1A (5′-AGAAGAAUUCAAGAGAUUCCGGAGG-3′/5′-CCUCCGGGAUCUCUUGAAUUCUUCU-3′) and negative control siRNA were purchased in a Stealth RNAi kit (Invitrogen). CRC cell lines were transfected with siRNA using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer’s protocols.

Invasion assay

The invasion assay was done using Transwell cell culture chambers (BD Biosciences) as described previously (21). Briefly, 5 × 10^5 cells were seeded in triplicate on the Matrigel-coated membrane. After 48 hours, cells that had invaded the underside of the membrane were fixed with 100% methanol and stained with 1% toluidine blue. Four microscopic fields were randomly selected for cell counting.

Generation of an adenoviral antisense construct to JMJD1A

The entire coding sequence of human JMJD1A was amplified by RT-PCR with primers 5′-GGTACCGCAGATTGGCTCACGCTCGGAG-3′ and 5′-CTCGAGTTAAGGTCTGCACACATGGTGCTC-3′ using mRNA prepared from KM12 cells. Adenoviral vectors containing

Fig. 1. Immunohistochemistry of CD34 and CA9 in liver metastasis. A, liver metastasis was double stained for CD34 as a vascular endothelial cell marker and CA9 as a hypoxic tumor cell marker. Tumor vessels (CD34; pink) decreased from the periphery to the central region. Conversely, CA9 expression in tumor cells (brown) increased from the periphery to the central region. Black arrows indicate that the intensity of CA9 staining was enhanced 80 μm from the tumor vessel. Magnification, ×400. B, scoring CD34 and CA9 staining. The CD34 score decreased significantly from the periphery to the central region (P < 0.01); conversely, the CA9 score increased significantly (P < 0.01). The details of each score are described in Materials and Methods. We then did LCM of CD34−CA9+ tumor cells around the central region and CD34+CA9− tumor cells around the periphery of CRC liver metastasis.
antisense JMJD1A were constructed using the AdEasy Adenoviral Vector System (Stratagene; ref. 21). Adenoviral recombination and preparation of infectious particles in HEK293 cells were described previously (22).

**Treatment of established tumor xenografts by intratumoral injection of an adenoviral antisense construct to JMJD1A**

Subcutaneous xenografts of the CRC cells DLD1 and HCT116 were established in nude mice (n = 5) by injection of 5 × 10⁶ cells. After 1 week, when the tumor size reached 100 to 200 mm³, Ad-Mock, adenoviral antisense (Ad-AS) construct to JMJD1A (1.0 × 10⁹ plaque-forming units per injection), and NaCl solution were injected into tumors on days 7, 9, 11, and 13.

**Statistical analysis**

Statistical analysis was done using the StatView 5.0 program (Abacus Concepts, Inc.). The Kaplan-Meier method was used to examine disease-free survival, and the log-rank test was used to determine statistical significance. A Cox proportional hazard model was used to assess the risk ratio with simultaneous contributions from several covariates. Statistical analysis was done using the Student’s t test or Fisher's exact test for categorical data and the Mann-Whitney U test for nonparametric data. Correlation significance was assessed using Pearson’s correlation coefficient test. Values of P < 0.05 denoted a statistically significant difference.

**Results**

**Immunohistochemistry of CD34 and CA9 in liver metastasis**

To identify in vivo hypoxic tumor cells, we first did double staining of CD34 and CA9 using 15 hepatic metastases of CRC (Fig. 1A). The CD34⁺ vascular endothelial cells (stained pink) significantly decreased from the periphery to the intermediate region to the central region of the metastasis. Contrast, CA9 expression in the tumor cells (stained brown) increased from the periphery to the central region (Fig. 1B). It was noteworthy that the intensity of CA9 staining became strong 80 μm from a tumor vessel in the intermediate region (Fig. 1A, middle), suggesting that CA9 is a sensitive marker for hypoxic tumor cells in hepatic metastasis of CRC.

**LCM and microarray analysis**

Using CA9 and CD34 as markers, CD34⁺CA9⁺ tumor cells in the periphery (Fig. 1A, left) and CD34⁺CA9⁺ tumor cells in the central region (Fig. 1A, right) were collected by LCM. After confirmation that high-quality RNA was derived from the tumor cells, 12 paired samples were subjected to microarray analysis.

The mean value of the fold induction in the central region relative to the periphery was calculated. Thirty genes with the greatest fold inductions are shown in Table 1, with the fold induction level ranging from 3.00 to 1.65. These 30 genes included well-known hypoxia-inducible genes, namely, egg-laying-defective nine homologue 3 (EGLN3), VEGF, endothelin-1 (EDN1), adrenomedullin (ADM), and peroxisome proliferator-activated receptor γ (PPARG). In addition, we found only a few reports that were related to hypoxia for prolyl-4-hydroxylase 1 (P4HA1), trefoil factor 3 (TFF3), endoplasmic reticulum oxido-reductin1-like (ERO1L), fatty acid binding protein (FABP1), and JMJD1A.

To investigate whether there were other novel hypoxia-inducible gene candidates, five colon cancer cell lines were subjected to microarray analysis after exposure to either hypoxic or normoxic conditions for 72 hours. We found that RNA levels were upregulated under hypoxic conditions in the genes that ranked 7th, 10th, 11th, 13th, 14th, 22nd, 23rd, 24th, and 29th in Table 1. The maximum induction of these genes was above 2.5-fold, which is comparable with the fold induction of known hypoxia-inducible genes (range, 1.2- to 8.3-fold).

**Selection of novel biomarkers for malignant primary CRC**

The well-known hypoxia-inducible genes VEGF, EDN1, EGLN3, TFF3, ADM, FABP1, and PPARG, listed in Table 1, were reported as poor prognostic factors for human malignancies (12, 23–32). To identify other genes that could serve as novel prognostic factors, we prospectively analyzed 214 CRC tissue samples using the same microarray chip; clinical data on disease recurrence were known for these samples. Our analysis indicated that JMJD1A (rank 9) and ADM (rank 21) were both significant prognostic markers for CRC. Although ADM was reported as a prognostic factor for ovarian cancer (30), JMJD1A has not been reported as a biomarker for human malignancies. Therefore, we subsequently focused on JMJD1A. A significant linear correlation of JMJD1A mRNA levels between microarray and qRT-PCR was observed (r = 0.0004; Supplementary Fig. S1A). Disease-free survival curves also showed that a high level of JMJD1A mRNA was a significant predictor of a shorter disease recurrence rate (r = 0.0054; Supplementary Fig. S1B). Supplementary Table S3 summarizes univariate and multivariate analyses for disease-free recurrence in the 214 CRC patients.

**JMJD1A expression determined by qRT-PCR and immunohistochemistry**

qRT-PCR analysis indicated that induced JMJD1A expression was 2.52-fold higher in the central region of liver metastasis compared with the periphery (r = 0.0094; Fig. 2A). Immunohistochemistry revealed that JMJD1A expression in the tumor cells (stained brown) increased from the periphery to the central region (Fig. 2B). Notably, the intensity of JMJD1A staining became strong 80 μm from a tumor vessel; this was also the case with CA9 staining. An approximately 3- to 6-fold induction of JMJD1A RNA was measured by qRT-PCR in the majority of CRC cell lines examined (Fig. 2C).
Survival survey

qRT-PCR was done using 142 independent CRC samples obtained from other institutes. High expression of JMJD1A RNA (n = 71, cutoff: median value; Supplementary Table S1B) was a significant prognostic factor with regard to cancer-related survival (P = 0.0108; Table 2). When analyzed with several clinicopathologic parameters that were statistically significant by univariate analysis, such as lymph node metastasis (P < 0.0001), lymphatic invasion (P < 0.0001), venous invasion (P = 0.0013), and depth of tumor invasion (P = 0.0008; Table 2), multivariate Cox regression analysis revealed that JMJD1A expression remained an independent prognostic factor (P = 0.0139; Table 2). As for the relationship between JMJD1A expression and each clinicopathologic characteristic, no significant difference was noted for age, gender, tumor location, and other factors (Supplementary Table S1B).

Effects of JMJD1A expression on growth and invasion of CRC cells

To assess the potential relevance of JMJD1A as a therapeutic target, in vitro knockdown experiments were done. Western blot analysis showed a significant reduction in the JMJD1A protein after siRNA treatment (Fig. 3A). A significant growth inhibition was observed in siRNA-treated HCT116 and DLD1 cell lines (P < 0.05 for each; Fig. 3B). Furthermore, invasion assays indicated that siRNA treatment significantly decreased the number of invaded cells of the two CRC cell lines when compared with control treatments (P < 0.05 for each; Fig. 3C).

Treatment of established tumor xenografts with an adenoviral construct of antisense JMJD1A

Similar to siRNA treatment, Ad-AS JMJD1A significantly decreased in vitro cell growth of the two CRC cells at a multiplicity of infection (MOI) of 100 (P < 0.05 for

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each; Fig. 4A). Similar results were obtained at MOI of 50 and 25 in both cells (data not shown). Treatment of established tumor xenografts, derived from the two cell lines, with intratumoral injection (four times) of Ad-AS JMJD1A significantly inhibited in vivo tumor growth when compared with the control groups (P < 0.05 for each; Fig. 4B).

Discussion

JMJD1A was initially identified in a testis cDNA library in 1991 (33), but its biological function has not yet been clarified. Studies gradually uncovered the roles of JMJD1A. JMJD1A is a histone H3 Lys9 demethylase that causes transcriptional activation of certain downstream target genes (12–14), and it plays a role in embryonic stem cell differentiation (34) and spermatogenesis (13). Here, we report for the first time that JMJD1A could be an important prognostic factor for patients with CRC.

In a chronically hypoxic microenvironment, cancer cells undergo genetic and adaptive changes that allow them to become more clinically aggressive and to develop resistance to irradiation and chemotherapy (8, 9, 35). An efficient therapeutic strategy against those cell types is essential to overcome cancer, and we aimed to determine a novel molecular target that is induced under in vivo hypoxic conditions.
Fig. 2. Expression of JMJD1A in liver metastatic lesions. A, RT-PCR analysis indicated that JMJD1A expression had a 2.52-fold higher induction in the central region ($n = 12; P = 0.0094$). B, JMJD1A expression in tumor cells (brown) increased from the periphery to the central region. Black arrows indicate the tumor vessels. The intensity of JMJD1A staining was enhanced 80 μm from the tumor vessel. Magnification, ×200. C, quantitative analysis of JMJD1A gene expression in CRC cell lines under hypoxic conditions. In the majority of CRC cell lines, JMJD1A mRNA expression increased progressively under hypoxic conditions, with the maximum 3- to 6-fold induction at approximately 48 to 72 h. *, $P < 0.05$.

**Table 2. Survival analysis of JMJD1A**

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<td>Tumor dedifferentiation</td>
<td>0.5574</td>
<td></td>
</tr>
<tr>
<td>(moderate/poor/mucinous/well)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (≥68/&lt;68)</td>
<td>0.9504</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: mp, muscularis propria; ss, subserosa.
To find in vivo hypoxia-inducible genes, we studied liver metastases from CRC patients. The CD34+ vascular architecture displayed a unique graded decline from the periphery to the central region (Fig. 1B); thus, it was easy to distinguish the region where hypoxic cells were present. We used CA9, which regulates cellular pH and allows cells to survive under hypoxic conditions, as a guide for hypoxic response. The distance from a vessel to the CA9 expression in cancer cells was 80 μm in head and neck tumors, leading to a tissue pO2 of 1% (36). We also found that CA9 expression was strongly induced in tumor cells positioned approximately 60 to 100 μm from tumor vessels (Fig. 1A, middle), indicating that CA9 was a reliable biomarker for hypoxia in liver metastasis of CRC. In addition, the metastatic cells are thought to originate from a single proliferating cell (37, 38). The homogeneous cell population may be better suited for doing a reliable comparative gene expression study between cells supplied with an abundant flow of blood and those without. By contrast, primary cancer tissue consists of highly heterogeneous cells (39) and oxygen levels are typically heterogeneous within individual tumors (9).

To find novel hypoxia-inducible genes, the method of collecting liver metastasis samples is of particular importance. To avoid surgery-related hypoxia, we consecutively collected only patients who had partial liver resection, but not hepatic lobectomy or segmentectomy; the latter surgery usually requires ligation of the Glissonian branch to reveal a clear ischemic demarcation line on the surface of the liver. Moreover, we stored the liver tissue samples in OCT compound as soon as possible after removal by surgery, usually within 10 to 15 minutes. To assure that our collection method was effective enough to reduce surgery-associated hypoxia to a minimum, we did qRT-PCR before microarray analysis and examined whether well-known hypoxia-associated genes were upregulated in the central region of hepatic metastasis compared with the periphery region. We confirmed that the
angiogenesis-related genes, such as angiopoietin2 (ANG2), VEGF, epidermal growth factor receptor (EGFR), fibroblast growth factor 2 (FGF2), and inducible nitric oxide synthase (iNOS), and the metabolism-related genes, such as GLUT1, GLUT3, lactate dehydrogenase A (LDHA), and phosphoglycerate kinase 1 (PGK1; refs. 1, 10, 40), showed 1.4- to 3.3-fold higher RNA in 12 paired samples (data not shown; primer sequences are shown in Supplementary Table S2). Microarray analysis successfully identified genes highly induced by hypoxia in vivo. VEGF ranked 5th among 30,000 human genes; 10 genes among the top 30 were well-known or relatively newly identified hypoxia-inducible genes (Table 1). We further identified several novel hypoxia-inducible gene candidates. The presence of many known hypoxia-related genes and prognostic factors in the list of the top 30 candidate genes is in good compliance with our initial concept that, in vivo, hypoxia-related genes should exert rather malignant properties. Because hypoxic stress is involved in cell death and survival, energy preservation, angiogenesis, pH regulation, and glucose metabolism, our data may shed some light on many aspects of cancer biology. After screening the top 30 genes based on a prospective clinical follow-up study in which the primary CRC tissues were analyzed with the same DNA chip, we focused on JMJD1A and concluded by qRT-PCR that JMJD1A is a novel independent prognostic factor for CRC (Table 2).

To assess the prognostic value of JMJD1A, it is important to see its relation to chemotherapy. When we analyzed disease-free survival between a group receiving chemotherapy and one that did not in a prospective series of 214 CRC patients (Supplementary Table S1A), we found that although a tendency was noted ($P = 0.059$), no statistical difference was observed between the two groups.

Fig. 4. Effects of Ad-AS JMJD1A on the growth of CRC cells. A, Ad-AS JMJD1A at MOI of 100 significantly inhibited tumor cell growth when compared with the Mock control in DLD1 and HCT116 ($P < 0.05$ for both). Western blot showed that Ad-AS JMJD1A at 100 MOI decreased JMJD1A protein expression at 48 h in DLD1 and HCT116. Actin bands served as loading controls. B, treatment of established tumor xenografts with intratumoral injection of Ad-AS JMJD1A. Subcutaneous xenografts of CRC cells (HCT116 and DLD1) were established in nude mice ($n = 5$ for each group) by injection of $5 \times 10^6$ cells. After day 7, Ad-Mock, Ad-AS JMJD1A ($1.0 \times 10^9$ plaque-forming units per injection), and NaCl solution were injected into tumors and three more injections per tumor were applied on days 9, 11, and 13. Tumor size on day 30 was significantly smaller in the AS group when compared with the Mock control group in both cell types ($P < 0.05$ for both).
groups (Supplementary Table S3). However, it was of interest that high expression of JMJD1A was significantly predictive of a poor prognosis in the group receiving chemotherapy (P = 0.013), but not in the group that did not receive chemotherapy (data not shown). The findings suggest that JMJD1A may lessen the efficacy of chemotherapy, which is consistent with the concept that hypoxia often makes tumor cells resistant to chemotherapy (8, 9, 35).

Recently, JMJD1A was shown for the first time to be upregulated by hypoxia via HIF-1 (41). Although Wellman et al. observed JMJD1A induction by hypoxia up to 24 hours, our data showed that the JMJD1A expression level continued to increase after 24 hours, reaching a maximum level of induction (3- to 6-fold) at 48 to 72 hours in CRC cell lines. In this regard, our gene list, generated from constitutive in vivo expression analysis, may reflect the chronic cellular responses to hypoxia. We also showed by immunohistochemistry that JMJD1A was a sensitive biomarker for in situ hypoxic cells (Fig. 2). The expression pattern was consistent with the report that hypoxic tumor cells are generally >100 μm away from functional blood vessels (42).

In vitro mechanistic studies showed that knockdown of JMJD1A reduced tumor cell growth and invasion. We also found that decreased growth effects from JMJD1A inhibition could be observed, irrespective of the VEGF level, in the colon cancer cells through treatment with siRNA against VEGF. Furthermore, the therapeutic in vivo models using an adenovirus-mediated antisense strategy against JMJD1A showed that JMJD1A could be a promising therapeutic target in CRC. Epigenetic modifications can affect various characteristics of cancer cells, such as apoptosis, invasion, angiogenesis, and immune recognition (43); therefore, it is possible that JMJD1A has pleiotropic effects in CRC by restoring defective expression of certain genes. We also speculate that JMJD1A might be a universal prognostic factor in human carcinomas via histone modification because our prospective microarray analysis indicated that JMJD1A was associated with poor prognosis in gastric cancer and hepatocellular carcinoma (data not shown). Although many downstream targets should be clarified in the future, a recent study has shown that JMJD1A regulates the expression of ADM and growth and differentiation factor 15 (GDF15) by decreasing histone methylation of these promoters (44). The results are consistent with our findings that both JMJD1A and ADM are novel prognostic markers in CRC.

In conclusion, we showed that JMJD1A could be a sensitive biomarker for hypoxic tumor cells and a poor prognosis of CRC. Our data also suggest that JMJD1A may be a novel therapeutic target, especially against tumor cells in a hypoxic condition.

Disclosure of Potential Conflicts of Interest

There are no potential conflicts of interest in this study.

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References


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