

Comparative integromics on *JMJD1C* gene encoding histone demethylase: Conserved POU5F1 binding site elucidating mechanism of *JMJD1C* expression in undifferentiated ES cells and diffuse-type gastric cancer

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Abstract. Epigenetic modifications of genomic DNA and histones alter the chromatin structure to regulate the accessibility of transcription factors to the promoter or enhancer regions. In 2003, we identified and characterized JMJD1C (TRIP8) consisting of TRI8H1 domain with C2HC4-type zinc finger-like motif, TRI8H2 domain with thyroid hormone receptor β -binding region, and JmjC domain. JMJD1A (TSGA), JMJD1B (5qNCA) and JMJD1C with the common domain architecture are histone H3K9 demethylases implicated in the nuclear hormone receptor-based transcriptional regulation. Here, comparative integromics on *JMJD1C* gene is reported. JMJD1C variant 1, previously reported, consists of exons 1, 2 and 3-26, while JMJD1C variant 2 characterized in this study was transcribed from novel exon 1B located 5' to exon 3. Four human JMJD1C ESTs were transcribed from exon 1, while 14 human JMJD1C ESTs from exon 1B. All of 26 mouse *Jmjd1c* ESTs were transcribed from exon 1b. These facts indicate that JMJD1C variant 2 transcribed from exon 1B was the major transcript. Human JMJD1C variant 2 with TRI8H1, TRI8H2, and JmjC domains showed 85.7% total-amino-acid identity with mouse *Jmjd1c*. Human *JMJD1C* mRNA was expressed in undifferentiated embryonic stem (ES) cells, pancreatic islet, diffuse-type gastric cancer, and other tissues or tumors. Mouse *Jmjd1c* mRNA was expressed in fertilized egg, blastocyst, undifferentiated ES cells, embryonic germ cells, c-Kit⁺/Sca-1⁺/Lin⁻ hematopoietic stem cells, pancreatic islet, and other tissues. Comparative genomics

analyses revealed that binding sites for POU5F1 (OCT3/OCT4), AP-1, and bHLH transcription factors within the promoter region located 5' to exon 1B of human *JMJD1C* gene were conserved in chimpanzee, cow, mouse and rat *JMJD1C* orthologs. POU5F1-mediated expression of JMJD1C histone demethylase is implicated in the reactivation of silenced genes in undifferentiated ES cells, pancreatic islet, and diffuse-type gastric cancer.

Introduction

Embryogenesis generating whole body from fertilized egg in the cell autonomous manner is regulated by the network of transcription factors as well as by the epigenetic modifications of chromatin structure around the key genes (1-3). Cancer cells acquire malignant phenotypes during multi-stage carcinogenesis due to the accumulation of epigenetic changes and genetic alterations of cancer-associated genes (4-6). Epigenetics to investigate the epigenetic regulation during embryogenesis and carcinogenesis is the scientific frontier in the post-genome era.

Chromatin structure is composed of a nucleosome unit with 147 bp genomic DNA wrapped around the core histone octamer (7-9). Genomic DNA is modified through methylation of cytosine bases located 5' to a guanosine in a CpG dinucleotide. Histones are modified through methylation, acetylation, phosphorylation, ubiquitylation, sumoylation, and ADP-ribosylation. Hypermethylation of genomic DNA as well as methylation at Lysine (K) 9 residue of histone H3 (H3K9), H3K27 and H4K20 are associated with regions of transcriptionally silenced chromatin. On the other hand, hypomethylation of genomic DNA as well as methylation at histone H3K4, H3K36, H3K79 and acetylation at histone H3K9 are associated with regions of transcriptionally active chromatin. Epigenetic modifications of genomic DNA and histones alter the chromatin structure to regulate the accessibility of transcription factors to the promoter or enhancer regions.

In 2003, we identified and characterized JMJD1C (TRIP8) consisting of TRI8H1 domain with C2HC4-type zinc finger-like motif, TRI8H2 domain with thyroid hormone receptor β -binding region, and JmjC domain (10). JMJD1A (TSGA),

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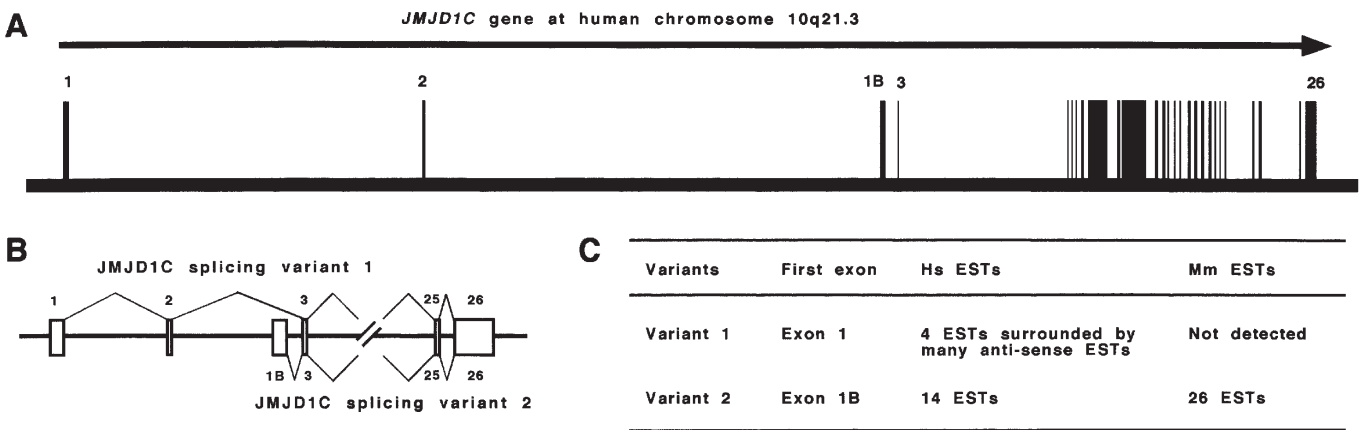


Figure 1. *JMJD1C* splicing variants. (A), Structure of *JMJD1C* gene. Exon 1B located within intron 2 was identified in this study. (B), Alternative promoter of *JMJD1C* gene. *JMJD1C* variant 1 previously reported consists of exons 1, 2 and 3-26, while *JMJD1C* variant 2 is transcribed from exon 1B. (C), Comparison between *JMJD1C* variants 1 and 2. *JMJD1C* variant 2 was the major transcript, which is evolutionarily conserved.

JMJD1B (5qNCA) and *JMJD1C* with the common domain architecture are *JMJD1* family histone demethylases (10-12).

Here, comparative integromics on *JMJD1C* gene is reported. *JMJD1C* variants 1 and 2 are splicing variants generated due to alternative promoter. *JMJD1C* variant 2 characterized in this study was the evolutionarily conserved major transcript. *JMJD1C* mRNA was expressed in undifferentiated embryonic stem (ES) cells, pancreatic islet, diffuse-type gastric cancer, and other tissues or tumors. Transcriptional mechanism of *JMJD1C* variant 2 in undifferentiated ES cells and diffuse-type gastric cancer will also be described.

Materials and methods

Identification and characterization of novel exon of human JMJD1C gene. Human genome sequences homologous to human *JMJD1C* RefSeq (NM_032776.1) were searched for with BLAST programs as described previously (13,14). Human ESTs homologous to genome sequence around the *JMJD1C* gene were next searched for to identify novel exon derived from the *JMJD1C* gene. *JMJD1C* isoform incorporating the novel exon was then characterized.

In silico expression analyses. Expressed sequence tags (ESTs) derived from human *JMJD1C* and mouse *Jmjd1c* genes were searched for using the BLAST programs as described previously (15,16). The sources of human ESTs were listed up for *in silico* expression analyses.

Comparative proteomics analyses. Amino-acid sequences of *JMJD1C* orthologs were aligned using Genetyx program. Domain architecture of *JMJD1C* orthologs was analyzed using RPS-BLAST program.

Comparative genomics analyses. Human genome sequence around the *JMJD1C* gene was compared with chimpanzee, cow, mouse, and rat genome sequences to identify evolutionarily conserved regions as described previously (17,18). Binding sites for transcription factors, such as TCF/LEF, POU5F1, SOX2 and NANOG were then searched for as described previously (19,20).

Results

Alternative promoters of human JMJD1C gene. BLAST programs using NM_032776.1 RefSeq revealed that human *JMJD1C* gene is located within the human genome sequences AC022022.10, AL607128.8, AL713895.8 and AL590502.12. We assembled these genome sequences around the human *JMJD1C* gene to construct the ‘*JMJD1C* genome contig’ of 310 kb in size. BLAST programs using the *JMJD1C* genome contig revealed that a novel exon was located between exons 2 and 3 of the *JMJD1C* gene (Fig. 1A). Human ESTs incorporating the novel exon were next searched for to characterize the novel exon. Ten ESTs were spliced from the novel exon to exon 3; however, no EST was spliced from exon 2 to the novel exon. Based on these facts, it was concluded that the novel exon was an alternative first exon. Therefore, the novel exon was designated exon 1B (Fig. 1A).

JMJD1C transcript previously reported consists of exons 1, 2 and 3-26 (10), while *JMJD1C* transcript characterized in this study was transcribed from novel exon 1B located 5' to exon 3 (Fig. 1B). Because some exons between exons 3 and 26 were spliced out due to alternative splicing of the cassette splicing type, *JMJD1C* variants 1 and 2 were defined as *JMJD1C* transcripts starting from exons 1 and 1B, respectively.

BLAST programs revealed that 4 human *JMJD1C* ESTs were transcribed from exon 1, and that 14 human *JMJD1C* ESTs were transcribed from exon 1B (Fig. 1C). In addition, all of 26 mouse *Jmjd1c* ESTs were transcribed from exon 1b (Fig. 1C). Based on these facts, it was concluded that *JMJD1C* variant 2 transcribed from exon 1B was the major transcript.

Comparative proteomics on JMJD1C orthologs. Human NM_004241.2 RefSeq and mouse AK173162.1 cDNA were the representative full-length cDNAs corresponding to *JMJD1C* variant 2. NP_004232.2 amino-acid sequence translated from NM_004241.2 RefSeq was used as the representative human *JMJD1C* amino-acid sequence. Nucleotide position 312-7598 was translated into amino-acid sequence in AK173162.1 cDNA; however, nucleotide position 657-7598

Hs	MIVMNDQVLEPQNDVPSMVQMTFLDDVVHSLKGENIGITSRRRSRANQNVNAVSHYTRAQANSRPRAMNSQAAPVKQNTHQQQQQRSSIRPNKRKGSDDSSIPDEEKMKEEKYDYISRGE	120
Mm	MIVMNDQVLEPQNDVPSMVQMTFLDDVVHSLKGENIGITSRRRSRANQNTVTHGHYTRAQANSRPRAMNSQAAPVKQNTHQQQQQRSSIRPNKRKGSDDSSIPDEEKMKEEKYDCVSRGE	120

Hs	NPKGKNKHLNKKRRRPEDEKKNMRRRLTDNVDSFESSDSSENSNKRIDNSS-EQKPENELKNKNTSKINGEEGKPHNNEKAGEETLKNQPPWDQIQEDKKHEEAERKKSVDLTQLQE	239
Mm	NPKGKNKHVVTTKRRRPEEAERKLSMKRLRDNASDAESSDAESSKRVETSSSEMPPEYEPKNKVTSKVNGEEGQSAEEAGEETLIDTRPPWDQMQEDKNHNEGEKPKSTDHSLQD	240

Hs	DMIHSSSEQSTVSDHNSNDLLPQECNMDKTHMELLPEKEFVSRPPTPKCVIDITNDTNLEKVAQENSSTFGLQTLQKMDPNVSDSKHSIANAKFLETAKKDSQSWVSDVVKVDLTQSS	359
Mm	KMTLRSSSEQATVADHNSNDVQLQECNVENQRTVELLPKDRLVSRTPPKCVIDITNDTHSERAAQENIATFGLQTPENMDPNVSDSKH--SNARYLETAKQDCDQSWVSDVVKVDLTQSS	358

Hs	VTNASSGNDHLNMEKEK--VYSYISPLSAVSVMEKDLHKRSPPPETIKSKLNTSVDTHTKIKSSPSPEVVKPKITHSPDSVKSKATYVNSQATGERRLANKIEHELSCSFHPITRSTSL	477
Mm	VTNAPSGSKRDTEKERNHYVYSMSLSAVSVTEQDLHKRSPPPETIKAKLTTSVDTKAKSSSSPEVVKPKITHSPDSVKSKAAYGNSQAVGERRLANKIEHELSCSFHPVPTRGSA	478

Hs	ETTKSPLIIDKNEHFTVYRDPALIGSETGANHISPLSQHPFLHSSSHRTCLNPGTHHPALTPAPHLLAGSSSQTPPLPTINTHPLTSGPHHVAHVHPHLLPTVLPVGPVTASLLGGHPRLE	597
Mm	ETTKSPLIIDKNEHFTVYRDPALIGSETGANHISPLSQHPFLHSSSHRTCLNPGTHHPALTPGPHLLAGSTSQTPPLPTINTHPLTSGPHHVAHVHPHLLPTVLPVGPVTASLLGGHPRLE	598

Hs	SAHASLSHLALAHQQQQQLLQHQSPHLLGQAHPASYNQLGLYPIIWQYPNGTHAYSGGLPSSKWHVPENAVNAEASLRNNSPSPWLHQPTVTSADGIGLLSHIPVRSSAEHPHRPL	717
Mm	SAHASLSHLALAHQQQQQLLQHQSPHLLGQAHPASYNQLGLYPIIWQYPNGTHAYSGGLPSSKWHVPENAVNAEASLRNNSPSPWLHQPTVTSADGIGLLSHIPVRSSAEHPHRPH	718

Hs	KITAHSSPPLTKTLVDHHEKEERKAFMEPLRSVASTSAKNDLDLNRSGTGKDLHLRHVFDPVLNQLQRPQETGERLNKYKEEHRRILQESIDVAPFTTKIKGLEGERENYSRVASSS	837
Mm	KITVHSSPPLTKTLADHHEKEERKAFMEPLRSNASTSVKGDLDLNRSGAGKDLHLRHVFDPV-----RPPQETGERLNKYKEEHRRILQESIDVAPFTTKIKGHEVERENYSRVVPS	832

Hs	SSPKSHI IKQDMVERSVDLYKMKHSVPQSLPQSNYFTTLSNSVNEPPRSYPSKEVSNYIGDKQSNALAAAAANPQTLTSTFITSLSKPPPLIKHQPESEGLVGKIPHLPHQIASHSV	957
Mm	SSPKSHAIKQDKVDVRSVSEIYKMKHSVPQSLPQSNYFTTLSNSVNEPPRSYPSKEVSNYITEKQNNLSAT-ANPQTH-SFISLSKPPPLIKHQPESEGLVGKIPDLHPHQASHSV	950

Hs	TTFRNDRCRSPHTLTVSSINTLRSPALHRAPVFPPIHHSLEKESYSSLSPTPLTPVMPVNAVGGKQVQESQKPPPTLIPKPKDSQANFKSSSEQSLTEMWRPNNNLSKEKTEWHVEKSSG	1077
Mm	TTFRNDRCRSPHTLTVSSINTLRSPALHRAPVFPPIHHSLEKESYSSLSPTPLTPVMPVNAVGGKQVQESQKPPPTLIPKPKDSQANFKNSDQSLTEMWRPNNNLSKEKTEWHVEKSSG	1070

Hs	KLQAAMASVIVRPPSSSTKTSMPAMQLASKDRVRSERSAGAHKTDCLKLAEGETGRIILPNVNSDSVHTKSEKNFQAVSQGVPSSVMSAVNMTCKTDTVITSAADTTSVSSWGGSEV	1197
Mm	KSQAAVASVIVRPPSSSTKTVDSVPSVPLASKDRVCERSSSGANKTDYLPK-EAGETGRIILPNVNSLESAHVSEKNFEAVSQGNVPVSVMSAVNVVSTKADVF TSAATTTSVSSLSAET	1189

Hs	ISLSLNTILASTSSSECVSSKS-VSQPVAQKQECVSTTAPVTLASSKTGSVVQPSGSGFTTDFIHLKHKHAAALAAQYKSSNASETEPNAIKNQTLSASLPDSTVICSTINKANSVGN	1316
Mm	SYLSLNTISASTPFECTSSKSVSVQAQAQKCTVSTAVPGTTLACSKTGSVAVQPSGSGFTTDFIHLKHKHAAALAAQFNSSVSEAEINTVRNQTVAAASLPDSTMTCTASNAKISVGN	1309

Hs	GQASQTSQPNYHTLKKKAWLTHRSEEDKNTNKMENSGNSVSEI IKPCSVNLIASTSSDIQNSVDSKIIVDKYVKKDKVNRKAKRTYESGSESGDSDESSEKSEQRTKRPKPTKYKKQN	1436
Mm	GPAQSSQPNYHTLKKKAWLTHRSEEDKNTNKMENSGNSVSEI IKPCSVNLIASTSSDIENRADGRVADVIRGDEKVSRRKAKRTYESGSESGDSDESSEKSEQRTKRPKPTKYKKQN	1429

Hs	DLQKRKGEIEEDLKPNGLVRSRAKERSKLKLQSNSTGIPRSVLKDWKRVKLLKQTGESFLQDSDCEIGPNLQKCRECLIRSKKGEPAHSPVFCRFYFRRLSFKNGVVRIDGFS	1556
Mm	DLQKRKGEIEEDSKPNGLVRSRAKERSKLKLQSNSTGIPRSVLKDWKRVKLLKQTGESFLQDSDCEIGPNLQKCRECLIRSKKGEESTHSPVFCRFYFRRLSFKNGVVRIDGFS	1549

Hs	PDQYDDEAMSLWTHENFEDDELDTETSKYILDIIGDKFCQLVTSEKTLASWVKDAKIAWKRAVRGVREMCDAEATLFNIHWVCQKCGFVVCLDCYKAKERKSSRDKEYLAWMKCVKGQ	1676
Mm	PDQYDDEAMSLWTHENYEDDEVETSKYILDIIGDKFCQLVTSEKTLASWVKDAKIAWKRAVRGVREMCDAEATLFNVHVVCRKCGFVACLDCYKAKERKSSRDKEYLAWMKCVKGQ	1669

Hs	PHDHKLHMPQTQIIPGSLVTLDDLAMHTLREKYIGKSHCHCTNRQNLQVGNFPTMNGVSQ-----SQQNTTPPKSEKNGSSPESDVGTDNKLTPPESQSPHLWLADL	1778
Mm	PHDHKLHMPQTQIIPGSLVTLDDLAMHTLREKYIGKSHCHCTNRQNLQVGNFPTMNGVSQVQLQNVLHHSNKTSSVLSPESSQQNSPQSSQTNGNSSPGS-ASTDSRLTPPESQSPHLWLADL	1788

Hs	AEQKAREEKKENKELTLENQIKEREQDNSESPNGRTSPLVSQNNQGGSTLRDLTLTTAGKLKRVGSTDAGIAFAPVYSMGAPSSKSGRTMPNIIIDDIASVVENKIPPSKTSKINVKPEL	1898
Mm	AEQKSREEKQENKEFTLERIKEDGDQDASDPNGSTSPPASQSNQGGSTLRDLTLTTAGKLKRVGSTDAGIAFAPVYSMGTSKSGGRTMPNIIIDDIASVVENKIPPNKTSKINIKSEP	1908

Hs	KEEPEESIIISAVDENNKLYSDIPHSWICEKHLWLKDYKNSNWKLFKECWKQGPVAVSGVHKKMNIISLWKAESISLDFGDHQADLLNCKDSIISNANVKEFWDFGEVSKRKQNKSGE	2018
Mm	NEEPKSSIPATDESNSKYRDIIPHSWICQHLWLKDYKNSNWKLFKECWKQGPVAVSGVHKKMNIISLWKAESISLDFGDHQADLLNCKDSIISNANVKEFWDFGEVSKRKQNKSGE	2028

Hs	TVVLKLDKDWPSGEDEKTMMPARYEDLLKSLPLPEYCNPEGKFNLAHLPGFFVRPDLGPRLCISAYGVAAKDHDIGTTLNLHIEVSDVNVILVYVGTAKNGVLSKAGILKFKFEEDLDI	2138
Mm	TVVLKLDKDWPSGEDEKTMMPARYEDLLKSLPLPEYCNPEGKFNLAHLPGFFVRPDLGPRLCISAYGVAAKDHDIGTTLNLHIEASDVNVNVLYVGTAKNGVLSKAGILKFKFEEDLDV	2148

Hs	LRKRLKDSSEIPGALWHIYAGKDVKIREFLQISKEQGLEVLPEHDPIRDQSWYVNNKLRQLLLEYGVRTCTLIQFLGDAIVLPAGALHQVQNFHSCIQVTEDFVSPHELVEFHLTQ	2258
Mm	LRKILKDSSEIPGALWHIYAGKDVKIREFLQISKEQGLEVLPEHDPIRDQSWYVNNKLRQLLLEYGVRTCTLIQFLGDAIVLPAGTLHQVQNFHSCIQVTEDFVSPHELVEFHLTQ	2268

Hs	ELRLLEEINYYDDKLQVKNILYHAVKEMVRALKIHEDEVEDMEEN	2303
Mm	ELRLLEEINYYDDKLQVKNILYHAVKEMVRALKMHEDEVEDMEDT	2313

Figure 2. JMJD1C orthologs. Human JMJD1C variant 2 and mouse Jmjd1c are aligned. Amino-acid residues are numbered on the right. Conserved amino-acid residues are shown by asterisks below the alignment. TRI8H1 domain with C2HC4-type zinc finger-like motif, TRI8H2 domain with thyroid hormone receptor β (TR β)-binding region, and JmJC domain are shown by open boxes. Two bipartite nuclear localization signals are shown by double overlines. Human JMJD1C variant 2 shows 85.7% total-amino-acid identity with mouse Jmjd1c.

was translated to determine the representative mouse Jmjd1c amino-acid sequence in this study (Fig. 2). Human JMJD1C and mouse Jmjd1c showed 85.7% total-amino-acid identity.

TRI8H1 domain with C2HC4-type zinc finger-like motif, TRI8H2 domain with thyroid hormone receptor β (TR β)-binding region, JmJC histone demethylase domain, and two bipartite nuclear localization signals previously identified in human JMJD1C variant 1 (10) were well conserved between human JMJD1C variant 2 and mouse Jmjd1c (Fig. 2).

Expression profile of JMJD1C orthologs. Thirteen JMJD1C ESTs were derived from ES cells. Among these human JMJD1C ESTs, 10 were expressed in undifferentiated human ES cells. Eight Jmjd1c ESTs were derived from undifferentiated mouse ES cells, and 18 from mouse embryonic germ (EG) cells. In addition, one Jmjd1c EST was derived from mouse fertilized egg, and 14 from mouse blastocyst. These facts indicate that mammalian *JMJD1C* orthologs were expressed in pluripotent stem cells during early embryogenesis.

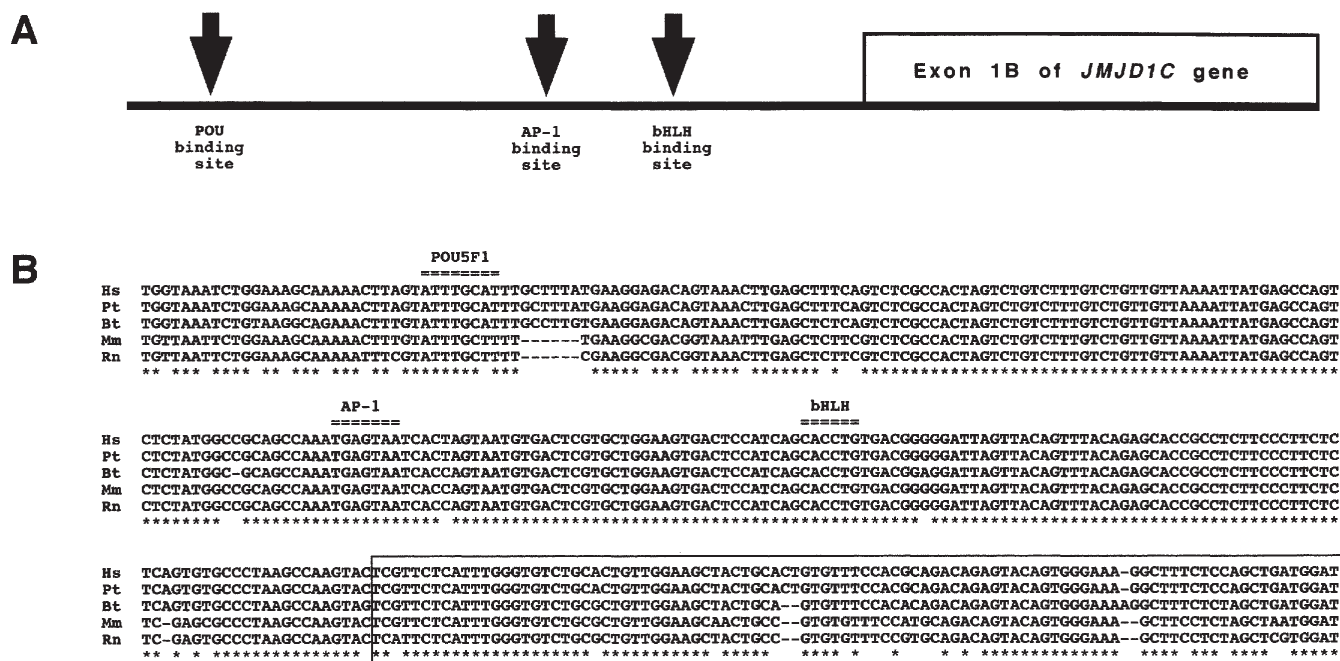


Figure 3. Promoter of the *JMJD1C* major transcript. (A), Schematic representation of promoter of *JMJD1C* major transcript transcribed from exon 1B. Binding sites for POU5F1, AP-1, and bHLH transcription factors are shown by arrows. (B), Alignment of mammalian *JMJD1C* promoter region. Hs, human; Pt, chimpanzee; Bt, cow; Mm, mouse; Rn, rat. Binding sites for POU5F1, AP-1, and bHLH transcription factors are conserved among promoter regions of mammalian *JMJD1C* orthologs.

Thirty-five *Jmjd1c* ESTs were derived from mouse hematopoietic stem cells. Among these mouse *Jmjd1c* ESTs, 24 were derived from c-Kit⁺, Sca-1⁺ and Lin⁻ population of mouse hematopoietic stem cells.

Mammalian *JMJD1C* orthologs were also expressed in adult tissues and tumors. Eight *JMJD1C* ESTs were derived from human islet, and three from human insulinoma. Six *Jmjd1c* ESTs were derived from mouse pancreatic islet. Four *JMJD1C* ESTs were derived from diffuse-type human gastric cancer.

Comparative genomics on *JMJD1C* promoters. Comparative genomics analyses revealed that the 5'-promoter for human *JMJD1C* variant 2 rather than variant 1 was well conserved in mouse (Fig. 3A). The 5'-promoter for human *JMJD1C* variant 2 was conserved not only in mouse, but also in chimpanzee, cow and rat (Fig. 3B).

Transcription factor-binding sites within the 5'-promoter for human *JMJD1C* variant 2 were next searched for based on Genetyx program, Match program, and manual annotation as previously described (19,20). POU-, AP-1-, and bHLH-binding sites were identified within the 5'-promoter for human *JMJD1C* variant 2. In addition, these POU-, AP-1-, and bHLH-binding sites were conserved in chimpanzee, cow, mouse, and rat *JMJD1C* orthologs (Fig. 3B).

Discussion

Comparative integromics on *JMJD1C* gene were investigated in this study. *JMJD1C* variant 1, previously reported, consists of exons 1, 2 and 3-26, while *JMJD1C* variant 2 characterized in this study was transcribed from novel exon 1B located 5' to exon 3. Four human *JMJD1C* ESTs were transcribed from

exon 1, while 14 human *JMJD1C* ESTs from exon 1B. All of 26 mouse *Jmjd1c* ESTs were transcribed from exon 1b (Fig. 1). These facts indicate that *JMJD1C* variant 2 transcribed from exon 1B was the major transcript.

JMJD1C belongs to the Jumonji family characterized by the JmjC domain. In 1995, Takeuchi *et al* cloned and characterized mouse *Jumonji* (*Jmj*) gene encoding a transcriptional repressor with JmjN and JmjC domains, which is the founding member of the Jumonji family (21). We identified and characterized *JMJD1C*, *JMJD2A*, *JMJD2B*, *JMJD2C* and *JMJD2D* genes encoding Jumonji family proteins in 2003 and 2004 (10,22). Because JmjC domain is implicated in histone demethylase activity, Jumonji family proteins play a key role during embryogenesis and carcinogenesis through the regulation of chromatin structure and gene expression (12,23).

Human *JMJD1C* variant 2 with TRI8H1, TRI8H2, and JmjC domains showed 85.7% total-amino-acid identity with mouse *Jmjd1c* (Fig. 2). *JMJD1C* orthologs share a common domain architecture with *JMJD1A* and *JMJD1B* (10). Hoog *et al* cloned and characterized mouse *Jmjd1a* as a male germ cell-specific transcript in 1991 (24). Yamane *et al* reported *JMJD1A*, using their in-house name JHDM2A, as a histone H3K9 demethylase implicated in the androgen receptor-mediated gene activation in 2006 (25). Hu *et al* cloned and characterized *JMJD1B* at human chromosome 5q35, which is deleted in myeloid leukemia in 2001 (26). *JMJD1C*, originally designated TRIP8, is a thyroid hormone receptor β -binding protein (10,11). *JMJD1* family members are histone demethylases, implicated in the transcriptional regulation of target genes for nuclear hormone receptors.

Human *JMJD1C* mRNA was expressed in undifferentiated ES cells, pancreatic islet, diffuse-type gastric cancer, and other tissues or tumors. Mouse *Jmjd1c* mRNA was expressed in

fertilized egg, blastocyst, undifferentiated ES cells, EG cells, c-Kit⁺/Sca-1⁺/Lin⁻ hematopoietic stem cells, and other tissues. Comparative genomics analyses revealed that binding sites for POU5F1, AP-1, and bHLH transcription factors within the promoter region locate 5' to exon 1B of human *JMJD1C* gene and were conserved in chimpanzee, cow, mouse and rat *JMJD1C* orthologs (Fig. 3). We recently reported preferential expression of *POU5F1* in undifferentiated ES cells, pancreatic islet, and diffuse-type gastric cancer (19). Together these facts indicate that POU5F1-mediated expression of JMJD1C histone demethylase is implicated in the reactivation of silenced genes in undifferentiated ES cells, pancreatic islet, and diffuse-type gastric cancer.

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