Binding of the JmjC Demethylase JARID1B to LSD1/NuRD Suppresses Angiogenesis and Metastasis in Breast Cancer Cells by Repressing Chemokine CCL14

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Abstract

JARID1B is a member of the JmjC/ARID family of demethylases that specifically demethylates tri- and di-methylated forms of histone H3 lysine 4 (H3K4) that are associated with active genes. JARID1B expression is dysregulated in several cancers in which it has been implicated, but how it might affect tumor progression is unclear. In this study, we report that JARID1B is a physical component of the LSD1/NuRD complex that functions in transcriptional repression. JARID1B and LSD1 acted in a sequential and coordinated manner to demethylate H3K4. A genome-wide transcriptional analysis revealed that among the cellular signaling pathways targeted by the JARID1B/LSD1/NuRD complex is the CCL14 chemokine pathway of cell migration and angiogenesis. JARID1B repressed the expression of CCL14, an epithelial derived chemokine, suppressing the angiogenic and metastatic potential of breast cancer cells in vivo. Our findings indicate that CCL14 is a critical mediator of the JARID1B/LSD1/NuRD complex in regulation of angiogenesis and metastasis in breast cancer, identifying a novel potential therapeutic target for breast cancer intervention. Cancer Res; 71(21); 6899–908. © 2011 AACR.

Introduction

JARID1B (KDM5B/PLU-1/RBP2-H1) is a member of the ARID (AT-rich DNA interaction domain) domain containing JmjC family of demethylases that specifically targets histone H3 lysine 4 (H3K4; ref. 1). JARID1B was originally isolated as a gene that was overexpressed in breast carcinomas (2). Subsequently, JARID1B dysregulation has been reported in several types of solid tumors, including prostate cancer (3), melanoma (4–7), and bladder cancer (8). Surprisingly, however, mechanistic studies on the role of JARID1B in different types of cancer have yielded inconsistent and even confusing results (1, 3, 5, 7–10). Therefore, the mechanistic role of JARID1B in tumor progression remains an intriguing topic. Interestingly, it becomes evident that the expression of JARID1B is dynamically regulated and might be associated with tumor grades. For example, in melanocytic tumors, JARID1B is highly expressed in benign nevi. However, in aggressive primary melanomas and melanoma metastases, the expression of JARID1B is down-regulated or even lost (4). Given its downregulation in aggressive primary tumors and metastases (4), the role, if any, of JARID1B in tumor angiogenesis and metastasis becomes an important question.

Here, we report that JARID1B is a component of the LSD1/NuRD complex. We showed that JARID1B and LSD1 act in a sequential manner and coordinated fashion in H3K4 demethylation. We showed that the JARID1B/LSD1/NuRD complex suppresses the angiogenic and metastatic potential of breast cancer cells by repressing the expression of an epithelial derived chemokine, CCL14.

Materials and Methods

Histone peptide binding assay

Five micrograms of purified proteins were incubated with 0.2 μg of biotinylated histone peptide in 100 μL of the binding buffer [50 mmol/L Tris–HCl (pH 7.5), 150 mmol/L NaCl, 0.05% NP-40, and 0.3 mg/mL bovine serum albumin] overnight at 4°C. Protein–peptide complexes were pulled down with streptavidin beads, washed 5 times, and subjected to Western blot analysis using anti-GST or other specific antibodies.
of 1 to 2 μg/μL nuclei. To generate nucleosomal material, digests were conducted by adding 1 volume of 2 × MNase buffer [50 mmol/L KCl, 8 mmol/L MgCl₂, 2 mmol/L CaCl₂, and 100 mmol/L Tris-HCl (pH 7.4)] and 50 to 500 μg/mL MNase. The reaction was incubated for 10 minutes at 37°C and stopped by adding EDTA to a final concentration of 10 mmol/L. The mononucleosomes were then purified by sucrose gradient assay.

Nucleosome binding assay

Five micrograms of purified proteins were incubated with 2 μg of mononucleosomes in the binding buffer [50 mmol/L Tris-HCl (pH 8.0), 150 mmol/L NaCl, 0.1% NP-40, 10 μmol/L ZnCl₂, and 1 mmol/L DTT] overnight at 4°C. Nucleosome binding assays were performed by incubating the bulk histones with antibodies against the corresponding components of the LSD1/NuRD complex, total proteins from HeLa cells, or JARID1B, followed by immunoblotting with antibodies against LSD1, MTA1, MTA2, MTA3, HDAC1, and HDAC2 (Fig. 1D).

To further substantiate the in vivo association of JARID1B and the LSD1/NuRD complex, protein fractionation experiments were carried out by fast protein liquid chromatography (FPLC; ref. 16). The results showed that the native JARID1B from HeLa cells eluted with an apparent molecular mass much greater than that of the monomeric protein. JARID1B immunoreactivity was detected in chromatographic fractions with a relatively symmetrical peak centered between approximately 667 and 2,000 kDa (Fig. 1E). Significantly, the elution pattern of JARID1B was clearly coeluted with the LSD1/NuRD complex proteins (Fig. 1E). Moreover, the chromatographic profiles of JARID1B and the LSD1/NuRD complex were compatible with their associated enzymatic activities. Specifically, histone deacetylation (HDAC) activity as well as histone demethylation (HDM) activities in both H3K4me2 and H3K4me3 were detected when the corresponding chromatographic fractions were incubated with the bulk histones and analyzed by Western blotting with antibodies against H3ac, H3K4me2, or H3K4me3 (Fig. 1E).

To further document the in vivo interaction between JARID1B and the LSD1/NuRD complex, we next used GST pull-down experiments to analyze the molecular detail involved in this interaction. Limited by the difficulty of purifying proteins with a high molecular weight from bacteria, these experiments were carried out with several GST-fused JARID1B fragments: JARID1B-N, which encompasses amino acids 1 to 721, and JARID1B-C, which spans amino acids 722 to 2,145 and harbors 2 PHD (plant homeodomain) domains and with in vitro transcribed/translated Mi-2, LSD1, MTA1, MTA2, MTA3, HDAC1, and HDAC3 (Fig. 2A); or with GST-fused MBΔ3, RbAp46, RbAp48, HDAC1, or HDAC2 and in vitro transcribed/translated JARID1B (Fig. 2B). These experiments revealed that JARID1B was able to interact with LSD1, HDAC1, and MBΔ3 in vitro. Considering the fact that LSD1, HDAC1, and MBΔ3 all also exist in other protein complexes (17–20), interaction of JARID1B with the multiple subunits of the LSD1/NuRD complex is logical in terms of its specific assembly into the JARID1B/LSD1/NuRD complex.

Functional coordination between JARID1B and LSD1 in H3K4 demethylation

As mentioned above, JARID1B has 3 PHD domains which have been characterized as one of the major functional modules for recognizing methylated histones (21, 22). Indeed, 2 other members of the JARID1 family, JARID1A and JARID1C, have been shown to be able to bind histone tails directly via their PHD domains (23, 24). On the contrary, it is interesting to
Figure 1. JARID1B is physically associated with the LSD1/NuRD Complex. A, immunopurification of JARID1B-containing protein complexes. Cellular extracts from HeLa cells stably expressing vector or FLAG-JARID1B were immunopurified with anti-FLAG affinity columns and eluted with FLAG peptides. The eluates were resolved by SDS-PAGE and silver stained. The protein bands were retrieved and analyzed by mass spectrometry. B, Western blotting analysis of the identified proteins in the purified fractions using antibodies against the indicated proteins. C, the JARID1B-containing protein complex purified from HeLa cells was incubated with calf thymus bulk histones in HDIM or HDAC assay buffer and analyzed by Western blotting using antibodies against the indicated histone marks or proteins. D, association of JARID1B with the LSD1/NuRD complex in HeLa cells (left) and in MCF-7 cells (right) by co-IP assays. Whole-cell lysates were immunoprecipitated with antibodies against the indicated proteins (JARID1B, left; NuRD components, right). Immunocomplexes were then immunoblotted (IB) using antibodies against the indicated proteins (NuRD components, left; JARID1B, right). E, cofractionation of JARID1B and the LSD1/NuRD complex by FPLC. Cellular extracts from HeLa cells were fractionated on Superose 6 size exclusion columns. Chromatographic elution profiles and immunoblotting analysis of the chromatographic fractions are shown. The elution positions of calibration proteins with known molecular masses (kDa) are indicated, and an equal volume from each fraction was analyzed.
note that LSD1 does not possess any currently known histone recognition modules in its structure. Rather, it is believed that LSD1 is presented to methylated histones via its association with other proteins that do possess histone recognition modules (15, 19). To test the hypothesis that JARID1B and LSD1 exert their catalytic activities sequentially to erase the methyl marks on H3K4, we first carried out peptide binding assays to examine the binding of JARID1B and LSD1 to H3K4me3, H3K4me2, and H3K4me1 in vitro. In these experiments, GST-fused JARID1B-N, JARID1B-C, or LSD1 were purified and incubated with histone H3 peptides that were methylated at different lysine residues and at different degrees, and the peptide-bound proteins were analyzed by Western blotting. As shown in Fig. 3A, JARID1B-C exhibited a strong affinity toward H3K4me3 peptides, whereas neither JARID1B-N nor LSD1 were able to bind H3K4me3 peptides. On the other hand, LSD1 was detected to bind H3K4me3 peptides, and JARID1B-C was found to bind H3K4me2 and H3K4me1 peptides (Fig. 3B). In agreement with these observations, incubation of the H3K4 peptides with HeLa cell lysates resulted in detection of LSD1 on H3K4me3 and of JARID1B on H3K4me2 and H3K4me1 peptides (Fig. 3B). Consistent with the binding pattern of JARID1B and LSD1 on H3K4 peptides, histone demethylase activity assays indicated that, although LSD1 alone did not lead to a decrease in H3K4me3 level or increases in H3K4me2 and H3K4me1 levels (Fig. 3C), when JARID1B was combined with LSD1, a much more dramatic decrease in H3K4me3 level and much more pronounced increase in H3K4me2 and H3K4me1 levels were observed (Fig. 3C). We believe that the increased level of H3K4me2 and H3K4me1 could be due to the possibility that at the point when the experiments were conducted, H3K4me3 demethylation is more efficient than H3K4me2 and H3K4me1 demethylation, when JARID1B and LSD1 work together to demethylate H3K4 hypermethylation. These results support the proposition that JARID1B and LSD1 act sequentially and in coordination to remove the methyl moieties on H3K4.

Next, we tested the functional connection between JARID1B and LSD1 in the context of chromatin. Mononucleosomes were isolated from HeLa cells and were incubated with GST-fused JARID1B-N, JARID1B-C, or LSD1 with WDR5 as a positive control. The bound materials were then analyzed by Western blotting using antibodies against H3, H3K4me3, or H3ac. The results indicated that JARID1B-C, but not JARID1B-N or LSD1, was able to bind nucleosomes and H3K4me3 was highly enriched (Fig. 3D). However, in the presence of GST-removed JARID1B-C, GST-LSD1 was able to bind nucleosomes. Consistent with this binding pattern, nucleosome demethylase activity assays indicated that, although JARID1B-C or LSD1 alone did not lead to a decrease in H3K4me3 level or increases in H3K4me2 and H3K4me1 levels, JARID1B-C and LSD1 in combination resulted in a marked and JARID1B-C dose-dependent decrease in H3K4me2 (Fig. 3E).
Identification of the transcriptional targets for JARID1B

To further investigate the functional association between JARID1B and the LSD1/NuRD complex and to explore the biological significance of this association, we analyzed the genome-wide transcriptional targets of the JARID1B/LSD1/NuRD complex using the Chromatin ImmunoPrecipitation–DNA Selection and Ligation (ChIP–DSL) approach. To this end, ChIP experiments were first conducted in MCF-7 cells with JARID1B antibodies. After ChIP, JARID1B-associated DNAs were amplified, labeled, and hybridized to AVIVA Hu20K arrays. Relative confidence prediction scores were generated by quantile normalization across each probe, followed by an analysis using a 2-state Hidden Markov model (25). The data from JARID1B antibodies were then analyzed with the data from antibodies against LSD1 that we had obtained previously (15) for overlapping promoters, and these promoters were considered to be the targets of the JARID1B/LSD1/NuRD complex. These experiments identified a total of 238 different promoters targeted by the JARID1B/LSD1/NuRD complex. Detailed results of the ChIP–DSL experiments are deposited in GEO Datasets (accession ID: GSE14260 for LSD1 and GSE25214 for JARID1B) and are summarized in Supplementary File S2.

The genes that are regulated by these promoters were then classified into cellular signaling pathways with a P value cutoff of 10^{-3}. These analyses identified several cellular signaling pathways, including the mitogen-activated protein kinase pathway, focal adhesion pathway, and cell-cycle pathway, as well as the chemokine pathway, which are all critically involved in cell proliferation, survival, and migration (Supplementary Fig. S1A). The ChIP–DSL results were verified by quantitative real-time reverse transcription PCR (RT-PCR) analysis of the mRNA expression of selected genes representing each of the pathways that are targeted by the JARID1B/LSD1/NuRD complex in MCF-7 cells with JARID1B knockdown (Supplementary Fig. S1B).

As stated above, chemokines have been implicated in cell proliferation, survival, and migration programs. Notably, chemokine CCL14 was identified as being targeted by the JARID1B/LSD1/NuRD complex. Measurement of the mRNA expression of CCL14 by quantitative real-time RT-PCR in MCF-7 cells with JARID1B overexpression or JARID1B knockdown indicated that JARID1B overexpression was associated with decreased CCL14 expression, and JARID1B knockdown was associated with increased CCL14 expression (Fig. 4A). Consistent with these results, the level of CCL14 protein in
conditioned medium from of MCF-7 cells was decreased with JARID1B overexpression and increased with JARID1B knockdown (Fig. 4B), as measured by Western blotting with antibodies against CCL14.

To examine whether JARID1B represses the transcription of CCL14 in the capacity of the JARID1B/LSD1/NuRD complex, we carried out ChIP assays in MCF-7 cells with control IgG or with antibodies against JARID1B, Mi-2, LSD1, MTA3, HDAC1, and MBD3 to detect the occupancy of these proteins on the promoter of the CCL14 gene. These experiments revealed that JARID1B, as well as the other components of the NuRD complex, occupied the promoter of the CCL14 gene (CCL14 primer a) but not at approximately 2 kb upstream from the CCL14 promoter (CCL14 primer b; Fig. 4C). To further support the idea that JARID1B and the NuRD complex interact and exist in the same protein complex on the CCL14 promoter, sequential ChIP experiments were done. In these studies, soluble chromatin was first immunoprecipitated with antibodies against JARID1B, and the supernatants and immunoprecipitates were subsequently reimmunoprecipitated with antibodies against Mi-2 or vice versa. Results indicate that, in precipitates, the CCL14 promoter that was immunoprecipitated with antibodies against JARID1B could be reimmunoprecipitated with antibodies against Mi-2, whereas in the

Figure 4. CCL14 is the transcriptional target of the JARID1B/LSD1/NuRD complex. A, MCF-7 cells were transfected with JARID1B or JARID1B siRNA. Total RNAs were prepared and analyzed for CCL14 mRNA expression by quantitative real-time RT-PCR. B, MCF-7 cells were transfected with JARID1B or JARID1B siRNA, and the proteins in the MCF-7 medium were collected and analyzed for CCL14 protein expression by Western blotting. C, the recruitment of the JARID1B/LSD1/NuRD complex on the CCL14 promoter. ChIP assays were done in MCF-7 cells using control IgG, JARID1B, Mi-2, LSD1, MTA3, HDAC1, or MBD3 antibodies with CCL14 primer a or CCL14 primer b. The acetylation and methylation degrees on the promoter of CCL14 were detected when MCF-7 cells were transfected with control siRNA, JARID1B siRNA, or LSD1 siRNA using CCL14 primer a or CCL14 primer b. D, JARID1B and the NuRD complex exist in the same protein complex on the CCL14 promoter. Sequential ChIP experiments were done with JARID1B or Mi-2 antibodies with CCL14 primer a or CCL14 primer b.
supernatants, no detectable CCL14 promoter was reimmunoprecipitated with antibodies against Mi-2 (Fig. 4D). The same results held when the initial ChIP was done with antibodies against Mi-2: the CCL14 promoter could only be detected in precipitates and not in supernatants after Re-ChIP with antibodies against JARID1B.

To determine the functional significance of JARID1B/LSD1/ NuRD complex binding on the CCL14 promoter, H3K4 methylation and H3 acetylation levels at the CCL14 promoter were analyzed in MCF-7 cells transfected with control siRNA (27), JARID1B siRNA, or LSD1 siRNA (Fig. 4C). These experiments showed that, whereas loss-of-function of JARID1B was associated with increased levels of H3K4me3, H3K4me2, and H3ac, knockdown of LSD1 was accompanied by increased levels of H3K4me2, H3K4me1, and H3ac (Fig. 4C). No changes in methylation or acetylation were observed using CCL14 primer b (Fig. 4C). These results are consistent with the notion that both JARID1B and LSD1 are required to modify H3K4 from a hypermethylation state to a hypomethylation state on the promoter of target genes. These observations are also in agreement with reports by us and others that HDAC and HDM are interdependent in transcription regulation (14, 15, 19, 28), and they further support the in vivo existence of the JARID1B/ LSD1/NuRD complex.

JARID1B inhibits the metastatic and angiogenic potential of breast cancer cells

The identification of CCL14 as a transcriptional target for the JARID1B/LSD1/NuRD complex could mean that JARID1B regulates the angiogenesis and metastasis of breast cancer through transcriptional repression of CCL14. To investigate this hypothesis, we first examined whether JARID1B could affect the metastatic potential of breast cancer cells. For this purpose, MDA-MB-231 cells were infected with lentiviruses carrying JARID1B or JARID1B siRNA, and the impact of the gain-of-function and loss-of-function of JARID1B on the invasive potential of these cells was investigated using transwell invasion assays. These experiments showed that overexpression of JARID1B resulted in a more than 5-fold decrease in cell invasion, and JARID1B knockdown led to a 2-fold increase in cell invasion (Fig. 5A). In addition, the effect of JARID1B on the invasive potential of MDA-MB-231 cells was probably through the association of JARID1B with the NuRD complex, as overexpression of JARID1B and knockdown of the expression of Mi-2 resulted in a diminished JARID1B effect (Fig. 5A). Moreover, the effect of JARID1B on the invasive potential of MDA-MB-231 cells was suppressed by overexpression of CCL14 and was enhanced by CCL14 knockdown (Fig. 5A).

To investigate whether JARID1B could affect the angiogenic potential of breast cancer cells through the regulation of CCL14, we first carried out endothelial tube formation assays to investigate whether JARID1B repressed angiogenesis of endothelial cells in vitro. This assay is based on the ability of endothelial cells to form 3-dimensional capillary-like tubular structures when cultured on an extracellular matrix gel prepared from Engelbreth-Holm-Swarm tumor cells (29). Human umbilical vein endothelial cells (HUVECs) that were infected with lentiviruses carrying JARID1B or JARID1B siRNA or cultured with different conditioned media (CM) from MCF-7 that were infected with lentiviruses carrying JARID1B or JARID1B siRNA were added onto solidified extracellular matrix gels. After incubation, endothelial cell tube formation was examined under light microscopy and the number of tubes was counted. As shown in Fig. 5B, HUVECs that were overexpressing JARID1B or cultured in the CM, which were overexpressing JARID1B, formed significantly fewer tubes than those in the control or vector/ control siRNA (mock) group, whereas HUVECs with JARID1B knockdown or that which were cultured in the CM with JARID1B knockdown generated more tubes than the control siRNA or mock group. In addition, the effect of JARID1B on HUVECs was likely through the association of JARID1B with the NuRD complex, as overexpression of JARID1B and knockdown of the expression of Mi-2 resulted in a diminished JARID1B effect (Fig. 5B). Moreover, the inhibitory effect of JARID1B overexpression on the angiogenesis potential of HUVECs could be almost totally rescued by addition of exogenous CCL14 protein (Fig. 5B).

Next, we carried out in vivo chicken yolk sac membrane (YSM) assays in which gelatin sponges that had adsorbed suspensions of MCF-7 cells transfected with JARID1B or JARID1B siRNA were placed on top of the YSM. Microscopic counting of the numbers of blood vessels entering the sponge showed that eggs treated with JARID1B-depleted MCF-7 cells developed enriched blood vessels toward the implants in a “spoked-wheel” pattern, whereas eggs treated with JARID1B overexpressed MCF-7 cells formed significantly fewer numbers of blood vessels compared with the control (Fig. 5C). However, the inhibitory effect of JARID1B overexpression on blood vessel formation could be alleviated by addition of exogenous CCL14 (Fig. 5C).

We then employed a well-established in vivo angiogenesis model (30), the mouse Matrigel plug assays, to further investigate the angiogenesis-inhibitory effect of JARID1B in vivo. In this assay, BALB/c female mice were randomly divided into 5 groups, and the animals in each group (n = 4) were injected subcutaneously with either Matrigels only, or with Matrigels that were mixed with MCF-7 cells infected with lentiviruses carrying vector/control siRNA (mock), JARID1B, JARID1B siRNA, or JARID1B plus supplementation with CCL14, respectively. Seven days after injection, the mice were sacrificed and the Matrigel plugs were processed and stained with HE and Masson trichrome. Microscopic examination of Matrigel plugs revealed that overexpression of JARID1B was associated with a decreased number of endothelial cells in the plugs, compared with that in the mock group (Fig. 5D). However, plugs in overexpression of JARID1B, but supplemented with CCL14, exhibited a comparable or even higher endothelial cell number than that of the mock group. On the other hand, abundant endothelial cells, often organized to form blood vessels containing red blood cells, were present in JARID1B siRNA plugs, although only a few endothelial cells had invaded the plugs of mock and Matrigel only group (Fig. 5D). These observations strongly support the idea that JARID1B suppresses angiogenesis in vivo, possibly through the repression of CCL14.
Figure 5. JARID1B inhibits breast cancer cell angiogenesis. 

A, MDA-MB-231 cells were infected with lentiviruses carrying JARID1B, JARID1B siRNA, JARID1B plus CCL14 siRNA, or JARID1B plus CCL14. The cells were starved for 18 hours before cell invasion assays were done using Matrigel transwell filters. The invaded cells were stained and counted. Representative images from each group are shown. Each bar represents the mean ± SD for triplicate measurements. Protein expression in these experiments was examined by Western blotting using antibodies against the indicated proteins. 

B, HUVECs were infected with lentiviruses carrying JARID1B siRNA, JARID1B, JARID1B plus Mi-2 siRNA, or JARID1B plus CCL14 (top), or HUVECs cultured with different CM from MCF-7 that were infected with lentiviruses carrying vector/control siRNA (mock), JARID1B siRNA, JARID1B, JARID1B plus Mi-2 siRNA, or JARID1B plus CCL14 (bottom) were added onto solidified extracellular matrix. After incubation, endothelial cell tube formation was assessed under light microscopy and the tubes were counted. Each bar represents the mean ± SD for triplicate measurements. Representative images from each group are shown. 

C, in vivo YSM assays were done. MCF-7 cells were transfected with JARID1B, JARID1B siRNA, or JARID1B plus CCL14. Then sponges which were absorbed with MCF-7 cells were placed on the YSM on day 8 and observed on day 12. The numbers of blood vessels entering the sponge in each group were counted; representative images from each group are shown. Each bar represents the mean ± SD for triplicate measurements. 

D, in vivo mouse Matrigel plug assays. Matrigels only or Matrigels mixed with MCF-7 cells infected with lentiviruses carrying empty vector/control siRNA (mock), JARID1B, JARID1B siRNA, or JARID1B plus CCL14 were injected subcutaneously into 6-week-old BALB/c female mice (n = 4). Seven days after injection, the mice were sacrificed and the Matrigel plugs were removed for HE and Masson trichrome staining and examined by microscopy.
Discussion

Although LSD1 was the first histone demethylase identified (31, 32), the enzymatic mechanism by which this protein initiates demethylation reactions precludes its catalysis of H3K4me3 substrate. Given the prevalence of the trimethyl modification state of histones (33, 34), it is apparent that LSD1 must collaborate with additional enzymes to accomplish H3K4 demethylation. Surprisingly, there have not yet been any reports on a functional connection between LSD1, an H3K4me1- and H3K4me2-specific demethylase, and the JARID1 family of proteins, the H3K4me3-specific demethylases. In this article, by using affinity purification and mass spectrometric analysis, we found that JARID1B is physically associated with LSD1 and with components of the NuRD complex. In light of our previous finding that LSD1 is an integral component of the NuRD complex (15), we propose that JARID1B is also a subunit in the NuRD complex. The likelihood of a physical association of JARID1B with the LSD1/NuRD complex is supported by the results from several other molecular experiments, including co-IP, FPLC, and GST pull-down. Significantly, the physical association between JARID1B and LSD1 provides a plausible molecular mechanism for sequential and coordinated demethylation regulation of H3K4. Indeed, our data show that JARID1B and LSD1 are functionally connected in H3K4 demethylation. Our experiments support a model for the action of the JARID1B/LSD1/NuRD complex on target gene promoters in which H3K4me3 is recognized by JARID1B first, and the methyl groups are then sequentially removed by the joint enzymatic activities of JARID1B and LSD1 to bring these genes to a silenced state.

In addition to its overexpression in breast cancers, JARID1B dysregulation has been reported in several types of solid tumors including prostate cancer (3), melanoma (4–7), and bladder cancer (8). It has also been reported that, functionally, JARID1B plays an important role in the proliferative capacity of breast cancer cells through repression of tumor suppressor genes, including BRCA1 (1), and this functionality seems to be consistent with its overexpression in breast cancer. However, it has also been suggested that JARID1B itself has a tumor suppressive activity, partly, due to its binding and ability to stabilize hypophosphorylated pRB, leading to maintenance of pRB-mediated cell-cycle control (5). In agreement with this, it has been observed that the expression of JARID1B is lost in the majority of advanced and metastatic melanomas and in many melanoma cell lines (4, 7). In this article, we showed that JARID1B is able to suppress angiogenesis and metastasis, supporting the notion that JARID1B itself is a potential tumor suppressor. We showed that the expression of CCL14 is negatively regulated by JARID1B. We also showed that inhibition of CCL14 expression is associated with a suppression of the angiogenesis and metastasis of breast cancer. As chemokine targeting is showing promise in animal models of inflammation and autoimmune disease (35), it is logical to believe that manipulating the local chemokine network may offer therapeutic benefits in cancers. In this sense, our data suggesting CCL14 to be a potent promoter of breast cancer angiogenesis and metastasis may provide a potential new target for breast cancer intervention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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