Published in final edited form as:

Biochem Biophys Res Commun. 2010 October 22; 401(3): 412–416. doi:10.1016/j.bbrc.2010.09.068.

Histone demethylase JARID1B/KDM5B is a corepressor of TIEG1/KLF10

Joanna Kim^{1, #}, Sook Shin^{2, #}, Malayannan Subramaniam¹, Elizabeth Bruinsma¹, Tae-Dong Kim², John R. Hawse¹, Thomas C. Spelsberg¹, and Ralf Janknecht^{2, *}

¹ Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN 55905, USA

² Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

Abstract

JARID1B/KDM5B (jumonji AT-rich interactive domain 1B/lysine-specific demethylase 5B) is an enzyme that efficiently removes methyl residues from trimethylated lysine 4 on histone H3, a pivotal mark for active chromatin. TIEG1/KLF10 (transforming growth factor- β inducible early gene-1/Krüppel-like transcription factor 10) is a zinc-finger transcription factor that is involved in bone metabolism and exerts antiproliferative activity. Here, we found that TIEG1 interacts with JARID1B. In particular, the repression domains of TIEG1 bind to the C-terminus of JARID1B. Moreover, overexpression of JARID1B augments TIEG1 to repress transcription of Smad7, an inhibitor of the TGF- β (transforming growth factor- β) signaling pathway. Conversely, JARID1B knock-down leads to increased Smad7 mRNA levels. Thus, TIEG1 and JARID1B may cooperate to suppress tumorigenesis by enhancing TGF- β signaling. Notably, both TIEG1 and JARID1B are downregulated in melanomas, suggesting that they indeed cooperate physiologically. In conclusion, JARID1B is the first TIEG1 corepressor identified, explaining how TIEG1 represses transcription through inducing histone H3 lysine 4 demethylation, which may be important for TIEG1 function in both normal and cancer cells.

Keywords

cancer; corepressor; histone demethylase; Krüppel-like transcription factor; Smad7; transforming growth factor- β

1. Introduction

JARID1B (jumonji AT-rich interactive domain 1B), also known as lysine-specific demethylase 5B (KDM5B) or PLU-1, is a histone demethylase and member of the family of JmjC domain-containing (JMJD) proteins. It specifically removes methyl residues from tri-, di- and monomethylated lysine 4 on histone H3 [1–4]. Since trimethylated lysine 4 on histone H3 marks transcriptionally active genes [5], JARID1B expectedly represses gene transcription at various promoters, where to it is recruited either through protein-protein

*Corresponding author. University of Oklahoma Health Sciences Center, 975 NE 10th Street, BRC-1464, Oklahoma City, OK 73104. Phone: +1-405-271 2377. Fax: +1-405-271 3548. ralf-janknecht@ouhsc.edu.

[#]These authors contributed equally to this manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

interactions with DNA binding proteins or by virtue of its ARID domain that selectively binds to CG-rich DNA [1,6,7]. Northern blot analyses showed that JARID1B is expressed at low levels in adult tissues with the exception of testes. However, JARID1B expression is upregulated in many breast cancer cell lines and tumors, suggesting that JARID1B has tumor promoting activities [8,9]. Accordingly, knock-down of JARID1B in breast cancer cells led to slower growth, reduced ability to form colonies in soft-agar and decreased tumor volume upon injection into the mammary fat pad [1]. Similarly, JARID1B is overexpressed in bladder and lung tumors and stimulates the proliferation of respective tumor cells [10]. On the other hand, JARID1B is downregulated in malignant melanomas and may suppress tumor formation by modulating the retinoblastoma protein [11,12]. Thus, JARID1B appears to exert opposite functions dependent on the cell type, but unfortunately we have currently only limited mechanistic knowledge as to how JARID1B may do so.

Transforming growth factor- β (TGF- β) inducible early gene-1 (TIEG1), also called Krüppel-like transcription factor 10 (KLF10), was uncovered as a transcript upregulated by TGF- β in osteoblasts [13]. In breast carcinomas, TIEG1 expression is downregulated [14,15] suggesting that TIEG1 may be a novel tumor suppressor, and consistently TIEG1 is able to curb cell proliferation and induce apoptosis [13]. One way of how TIEG1 may exert its anti-tumor activity is by modulating TGF- β signaling. TGF- β leads to the intracellular activation of Smad2, Smad3 and Smad4, which can be counteracted by the inhibitory proteins, Smad6 and Smad7 [16]. Since TIEG1 represses the Smad7 promoter and also induces Smad2 transcription, TIEG1 may enhance the tumor suppressing effects of TGF- β [17,18]. Here, we have investigated if TIEG1 cooperates with JMJD proteins, including JARID1B, in reducing Smad7 promoter activity.

2. Materials and methods

2.1. Coimmunoprecipitations

Human 293T cells were transiently transfected by the calcium phosphate coprecipitation method [19,20] with indicated expression plasmids. Cells were harvested 36 h after transfection and immunoprecipitations performed as described [21,22]. Enhanced chemiluminescence was used to reveal tagged proteins after Western blotting [23].

2.2. GST pull-down assays

Fusions of JARID1B amino acids to GST (glutathione *S*-transferase) were produced in *E. coli* and affinity purified according to standard procedures [24]. GST-JARID1B fusion proteins were then bound to glutathione agarose and binding of Myc-tagged TIEG1 assessed essentially as described [25].

2.3. Luciferase assays

293T cells grown in 12-well plates were transiently transfected with the following plasmids: 0.2 μ g of Smad7-luciferase [17] or CMV-luciferase reporter [26], 2 μ g of pBluescript KS⁺, and indicated amounts of pEV-Flag-TIEG1, Flag-JARID1B or pEV3S vector control. 36 h after transfection, cells were lysed [27] and luciferase activities determined as described [28].

2.4. JARID1B knock-down

To downregulate JARID1B, shRNA targeting the sequence GCACCAAATTAGAGAGTCT was cloned into the retroviral expression vector pSIREN-RetroQ (Clontech). MDA-MB-231 cells were infected with respective retrovirus according to standard procedures and shRNA expressing cells selected with 1 μ g/ml puromycin [29].

2.5. RT-PCR

Human MDA-MB-231 cells were lysed in Trizol (Invitrogen) and total RNA isolated as described [30]. Reverse transcription and amplification of Smad7 cDNA was performed with the Access Quick RT-PCR system (Promega) utilizing the following PCR program [31]: 48°C for 45 min; 96°C for 2 min; 30 repeats (for Smad7) or 18 repeats (for GAPDH) of 95°C for 30 s, 55°C for 40 s, and 68°C for 40 s; followed by a final extension at 68°C for 4 min. GAPDH primers were described before [32], and Smad7-RT-for2 (5'-GGCTGTGTTGCTGTGAATCTTACG-3') and Smad7-RT-rev2 (5'-CTCTCGTCTTCTCCTCCCAGTATG-3') primers were employed to amplify a 291 bp product that was visualized on ethidium-stained agarose gels [33].

3. Results

3.1. TIEG1 interacts with JARID1B

To identify JMJD proteins that interact with TIEG1, we cloned 14 different JMJD cDNAs into an expression vector with an N-terminal 6Myc-tag and cotransfected these with Flag-tagged TIEG1 into 293T cells. Extracts obtained from transfected cells were challenged with anti-Myc antibodies and coprecipitated TIEG1 revealed by anti-Flag Western blotting (Fig. 1). We observed that TIEG1 most strongly coimmunoprecipitated with JARID1B, but not with the closely related JARID1C protein and also not with JMJD2A, JMJD2C, JMJD2D, JMJD1A, JMJD1B, UTX, JMJD5 and JMJD6. Moreover, TIEG1 coimmunoprecipitated to a lower degree with PHF2, KIAA1718, JHDM1A and JMJD4, yet we did not pursue these weak interactions any further. To confirm the interaction of JARID1B with TIEG1, we switched the tags on JARID1B and TIEG1. In this scenario, Flag-tagged JARID1B also coimmunoprecipitated with Myc-tagged TIEG1 (Fig. 2A). We conclude that JARID1B and TIEG1 form complexes *in vivo*.

3.2. Mapping of interaction domains

TIEG1 is a DNA binding zinc-finger protein, which possesses three N-terminal repression domains [13]. To determine which TIEG1 amino acids bind to JARID1B, we expressed either TIEG1's N-terminal repression domains or its C-terminal DNA binding domain with JARID1B. Coimmunoprecipitation assays revealed that TIEG1 amino acids 1–360, which encompass all three repression domains, interacted with JARID1B, whereas the DNA binding domain of TIEG1 did not (Fig. 2B). This implicates that one mechanism of action for the TIEG1 repression domains is the recruitment of JARID1B, which then removes active chromatin marks.

JARID1B encompasses several functional domains (Fig. 2C): the JmjN domain required for demethylase activity, the DNA binding ARID domain, the catalytic JmjC domain, a C5HC2 zinc-finger and three PHD (plant homeodomain) domains that may bind to methylated histone residues. To determine which domains in JARID1B mediate its interaction with TIEG1, we generated four GST-JARID1B fusion proteins spanning the whole JARID1B molecule. Interestingly, both JARID1B amino acids 801–1150 and 1151–1544 were capable of interacting with TIEG1, whereas most of the known functional domains (JmjN, ARID, JmjC, C5HC2 and N-terminal PHD domain) appear to be not involved in JARID1B binding (Fig. 2C). Thus, binding of TIEG1 is unlikely to obstruct the DNA binding or histone demethylase activity of JARID1B by occluding its access to DNA or histones.

3.3. Impact of JARID1B on TIEG1 function

Previously, it was shown that Smad7 is a target gene of TIEG1 [17]. Accordingly, TIEG1 expression in 293T cells led to a dose-dependent repression of Smad7 transcription, whereas a control CMV reporter gene was unaffected (Fig. 3A). Notably, the very low amount of 1

ng of TIEG1 expression plasmid already led to ~25% repression, and 5 ng of TIEG1 expression plasmid caused ~50% reduction of luciferase activity. If JARID1B is a cofactor of TIEG1, it should also repress Smad7 transcription. Indeed, JARID1B overexpression also caused repression of Smad7 transcription in a dose-dependent manner (Fig. 3B). Again, a control CMV reporter gene was unaffected up to 100 ng of JARID1B expression plasmid, showing that the JARID1B effect on Smad7 transcription was specific. Please note that the CMV reporter was slightly repressed by 400 ng of JARID1B expression plasmid, which is probably due to the ability of JARID1B to globally affect histone methylation and thereby lead to unspecific repression at higher JARID1B concentrations.

To assess whether TIEG1 and JARID1B cooperate in the repression of Smad7 transcription, we chose low levels of TIEG1 (1 ng) and JARID1B (15 ng) expression plasmids that individually led to only slight repression of Smad7 transcription and did not affect the CMV reporter (see Fig. 3A and 3B). When coexpressed, TIEG1 and JARID1B cooperated by reducing Smad7 transcription by 44%, whereas individually TIEG1 and JARID1B led to only 18% and 22% repression, respectively (Fig. 3C). These data demonstrate that JARID1B is a corepressor of TIEG1.

Finally, we also downregulated JARID1B in human MDA-MB-231 breast cancer cells to assess its impact on endogenous Smad7 transcription. Western blotting revealed that the JARID1B protein level was significantly reduced upon infecting MDA-MB-231 cells with retrovirus expressing JARID1B shRNA (Fig. 4A). RNA was isolated from these cells and RT-PCR performed to determine Smad7 mRNA levels. We observed that the Smad7 transcript level was enhanced upon knock-down of JARID1B (Fig. 4A), which is consistent with JARID1B being a corepressor of TIEG1.

4. Discussion

In this report, we have identified the JMJD protein JARID1B as a novel interaction partner of TIEG1. This provides an explanation of how TIEG1 can repress transcription through recruitment of a histone demethylase that removes one of the most important marks of active chromatin, trimethylated lysine 4 on histone H3. Interestingly, the closely related JARID1C protein as well as many other JMJD proteins did not interact with TIEG1, indicating a high degree of specificity in its ability to recruit JMJD proteins.

Our data show that JARID1B cooperates with TIEG1 in repressing the Smad7 promoter. The only other hitherto known TIEG1 target gene is osteoprotegerin [34], an important regulator of skeletal biology, and our data predict that JARID1B will contribute to its repression via TIEG1. Smad7 is an inhibitor of the intracellular TGF- β effectors, and accordingly TIEG1 and JARID1B may, through their repression of Smad7 transcription, enhance the tumor suppressing effects of TGF- β . Remarkably, JARID1B is downregulated in skin tumors [11], and we found that TIEG1 mRNA levels are decreased in melanomas similarly to JARID1B (Fig. 4B). This supports the notion that TIEG1 and JARID1B cooperate in repressing Smad7 transcription and thereby antagonize skin cancer development.

In contrast, JARID1B is upregulated in breast cancer, whereas TIEG1 is downregulated [8,9,14,15], pointing to opposite roles of these two proteins in breast carcinogenesis. However, both downregulation of TIEG1 and upregulation of JARID1B may still contribute to breast tumor formation through modulating Smad7 transcription. Early in tumorigenesis, cancer cells must escape the tumor suppressing activities of TGF- β to progress to the malignant stage, and downregulation of TIEG1 may help as it leads to enhanced Smad7 transcription, thus curtailing the tumor suppressing activities of TGF- β . However, TGF- β is

a double-edged sword in cancer as it also exerts tumor promoting effects in particular at later stages of tumorigenesis, for instance by inducing epithelial-mesenchymal transition or production of autocrine mitogens [16]. Thus, one may envision that JARID1B upregulation at later stages of breast tumorigenesis counteracts an earlier decrease of TIEG1 and leads to enhanced repression of Smad7 transcription, thereby allowing for the tumorigenic effects of TGF- β to occur in advanced breast cancer cells.

Apart from its role in cancer, TIEG1 has profound effects in bone metabolism and heart development. This is evidenced by the phenotype of TIEG1 knock-out mice that display osteopenia in females and hypertrophic cardiomyopathy in males [13]. Our study suggests that JARID1B may also cooperate with TIEG1 in bone and heart cells. If so, to-be-generated JARID1B knock-out mice should display some or all of the phenotypes of TIEG1 knock-out mice. Finally, TIEG1 and thus its corepressor JARID1B may cooperate in many other processes that are regulated by TIEG1, including allergic responses, atherosclerosis and circadian energy metabolism [35–37].

In conclusion, our study has identified the first interaction partner of TIEG1, whose function as a histone demethylase explains why TIEG1 is able to repress transcription in normal and cancer cells.

Acknowledgments

This work was generously supported by the Bruce and Martha Atwater Foundation and NIH grant R01DE014036. These funding sources had no role in the study design, data collection, data interpretation, writing of this report and the decision to submit this paper for publication.

Abbreviations

GST	glutathione <i>S</i> -transferase
JARID1B	jumonji AT-rich interactive domain 1B
JMJD	JmjC domain-containing
KDM5B	lysine-specific demethylase 5B
KLF10	Krüppel-like transcription factor 10
PHD	plant homeodomain
TGF-β	transforming growth factor- β
TIEG1	TGF- β inducible early gene-1

References

1. Yamane K, Tateishi K, Klose RJ, Fang J, Fabrizio LA, Erdjument-Bromage H, Taylor-Papadimitriou J, Tempst P, Zhang Y. PLU-1 is an H3K4 demethylase involved in transcriptional repression and breast cancer cell proliferation. *Mol Cell*. 2007; 25:801–812. [PubMed: 17363312]
2. Christensen J, Agger K, Cloos PA, Pasini D, Rose S, Sennels L, Rappsilber J, Hansen KH, Salcini AE, Helin K. RBP2 belongs to a family of demethylases, specific for tri- and dimethylated lysine 4 on histone 3. *Cell*. 2007; 128:1063–1076. [PubMed: 17320161]
3. Seward DJ, Cubberley G, Kim S, Schonewald M, Zhang L, Tripet B, Bentley DL. Demethylation of trimethylated histone H3 Lys4 in vivo by JARID1 JmjC proteins. *Nat Struct Mol Biol*. 2007; 14:240–242. [PubMed: 17310255]
4. Xiang Y, Zhu Z, Han G, Ye X, Xu B, Peng Z, Ma Y, Yu Y, Lin H, Chen AP, Chen CD. JARID1B is a histone H3 lysine 4 demethylase up-regulated in prostate cancer. *Proc Natl Acad Sci USA*. 2007; 104:19226–19231. [PubMed: 18048344]

5. Martin C, Zhang Y. The diverse functions of histone lysine methylation. *Nat Rev Mol Cell Biol.* 2005; 6:838–849. [PubMed: 16261189]
6. Tan K, Shaw AL, Madsen B, Jensen K, Taylor-Papadimitriou J, Freemont PS. Human PLU-1 has transcriptional repression properties and interacts with the developmental transcription factors BF-1 and PAX9. *J Biol Chem.* 2003; 278:20507–20513. [PubMed: 12657635]
7. Scibetta AG, Santangelo S, Coleman J, Hall D, Chaplin T, Copier J, Catchpole S, Burchell J, Taylor-Papadimitriou J. Functional analysis of the transcription repressor PLU-1/JARID1B. *Mol Cell Biol.* 2007; 27:7220–7235. [PubMed: 17709396]
8. Lu PJ, Sundquist K, Baekstrom D, Poulsom R, Hanby A, Meier-Ewert S, Jones T, Mitchell M, Pitha-Rowe P, Freemont P, Taylor-Papadimitriou J. A novel gene (PLU-1) containing highly conserved putative DNA/chromatin binding motifs is specifically up-regulated in breast cancer. *J Biol Chem.* 1999; 274:15633–15645. [PubMed: 10336460]
9. Barrett A, Madsen B, Copier J, Lu PJ, Cooper L, Scibetta AG, Burchell J, Taylor-Papadimitriou J. PLU-1 nuclear protein, which is upregulated in breast cancer, shows restricted expression in normal human adult tissues: a new cancer/testis antigen? *Int J Cancer.* 2002; 101:581–588. [PubMed: 12237901]
10. Hayami S, Yoshimatsu M, Veerakumarasivam A, Unoki M, Iwai Y, Tsunoda T, Field HI, Kelly JD, Neal DE, Yamaue H, Ponder BA, Nakamura Y, Hamamoto R. Overexpression of the JmjC histone demethylase KDM5B in human carcinogenesis: involvement in the proliferation of cancer cells through the E2F/RB pathway. *Mol Cancer.* 2010; 9:59. [PubMed: 20226085]
11. Roesch A, Becker B, Meyer S, Wild P, Hafner C, Landthaler M, Vogt T. Retinoblastoma-binding protein 2-homolog 1: a retinoblastoma-binding protein downregulated in malignant melanomas. *Mod Pathol.* 2005; 18:1249–1257. [PubMed: 15803180]
12. Roesch A, Becker B, Schneider-Brachert W, Hagen I, Landthaler M, Vogt T. Re-expression of the retinoblastoma-binding protein 2-homolog 1 reveals tumor-suppressive functions in highly metastatic melanoma cells. *J Invest Dermatol.* 2006; 126:1850–1859. [PubMed: 16645588]
13. Subramaniam M, Hawse JR, Johnsen SA, Spelsberg TC. Role of TIEG1 in biological processes and disease states. *J Cell Biochem.* 2007; 102:539–548. [PubMed: 17729309]
14. Subramaniam M, Hefferan TE, Tau K, Peus D, Pittelkow M, Jalal S, Riggs BL, Roche P, Spelsberg TC. Tissue, cell type, and breast cancer stage-specific expression of a TGF-beta inducible early transcription factor gene. *J Cell Biochem.* 1998; 68:226–236. [PubMed: 9443078]
15. Reinholz MM, An MW, Johnsen SA, Subramaniam M, Suman VJ, Ingle JN, Roche PC, Spelsberg TC. Differential gene expression of TGF beta inducible early gene (TIEG), Smad7, Smad2 and Bard1 in normal and malignant breast tissue. *Breast Cancer Res Treat.* 2004; 86:75–88. [PubMed: 15218362]
16. Massague J. TGFbeta in Cancer. *Cell.* 2008; 134:215–230. [PubMed: 18662538]
17. Johnsen SA, Subramaniam M, Janknecht R, Spelsberg TC. TGFbeta inducible early gene enhances TGFbeta/Smad-dependent transcriptional responses. *Oncogene.* 2002; 21:5783–5790. [PubMed: 12173049]
18. Johnsen SA, Subramaniam M, Katagiri T, Janknecht R, Spelsberg TC. Transcriptional regulation of Smad2 is required for enhancement of TGFbeta/Smad signaling by TGFbeta inducible early gene. *J Cell Biochem.* 2002; 87:233–241. [PubMed: 12244575]
19. Dowdy SC, Mariani A, Janknecht R. HER2/Neu- and TAK1-mediated up-regulation of the transforming growth factor beta inhibitor Smad7 via the ETS protein ER81. *J Biol Chem.* 2003; 278:44377–44384. [PubMed: 12947087]
20. Shin S, Janknecht R. Diversity within the JMJD2 histone demethylase family. *Biochem Biophys Res Commun.* 2007; 353:973–977. [PubMed: 17207460]
21. Shin S, Janknecht R. Activation of androgen receptor by histone demethylases JMJD2A and JMJD2D. *Biochem Biophys Res Commun.* 2007; 359:742–746. [PubMed: 17555712]
22. Kim TD, Shin S, Janknecht R. Repression of Smad3 activity by histone demethylase SMCX/JARID1C. *Biochem Biophys Res Commun.* 2008; 366:563–567. [PubMed: 18078810]
23. Janknecht R. Regulation of the ER81 transcription factor and its coactivators by mitogen- and stress-activated protein kinase 1 (MSK1). *Oncogene.* 2003; 22:746–755. [PubMed: 12569367]

24. Goel A, Janknecht R. Concerted activation of ETS protein ER81 by p160 coactivators, the acetyltransferase p300 and the receptor tyrosine kinase HER2/Neu. *J Biol Chem*. 2004; 279:14909–14916. [PubMed: 14747462]
25. Wu J, Janknecht R. Regulation of the ETS transcription factor ER81 by the 90-kDa ribosomal S6 kinase 1 and protein kinase A. *J Biol Chem*. 2002; 277:42669–42679. [PubMed: 12213813]
26. Knebel J, De Haro L, Janknecht R. Repression of transcription by TSGA/Jmjd1a, a novel interaction partner of the ETS protein ER71. *J Cell Biochem*. 2006; 99:319–329. [PubMed: 16619273]
27. Papoutsopoulou S, Janknecht R. Phosphorylation of ETS transcription factor ER81 in a complex with its coactivators CREB-binding protein and p300. *Mol Cell Biol*. 2000; 20:7300–7310. [PubMed: 10982847]
28. Goueli BS, Janknecht R. Upregulation of the catalytic telomerase subunit by the transcription factor ER81 and oncogenic HER2/Neu, Ras, or Raf. *Mol Cell Biol*. 2004; 24:25–35. [PubMed: 14673140]
29. Shin S, Janknecht R. Concerted activation of the Mdm2 promoter by p72 RNA helicase and the coactivators p300 and P/CAF. *J Cell Biochem*. 2007; 101:1252–1265. [PubMed: 17226766]
30. Shin S, Kim TD, Jin F, van Deursen JM, Dehm SM, Tindall DJ, Grande JP, Munz JM, Vasmatazis G, Janknecht R. Induction of prostatic intraepithelial neoplasia and modulation of androgen receptor by ETS variant 1/ETS-related protein 81. *Cancer Res*. 2009; 69:8102–8110. [PubMed: 19789348]
31. Goel A, Janknecht R. Acetylation-mediated transcriptional activation of the ETS protein ER81 by p300, P/CAF, and HER2/Neu. *Mol Cell Biol*. 2003; 23:6243–6254. [PubMed: 12917345]
32. Shin S, Bosc DG, Ingle JN, Spelsberg TC, Janknecht R. Rcl is a novel ETV1/ER81 target gene upregulated in breast tumors. *J Cell Biochem*. 2008; 105:866–874. [PubMed: 18726892]
33. Shin S, Rossow KL, Grande JP, Janknecht R. Involvement of RNA helicases p68 and p72 in colon cancer. *Cancer Res*. 2007; 67:7572–7578. [PubMed: 17699760]
34. Subramaniam M, Hawse JR, Bruinsma ES, Grygo SB, Cicek M, Oursler MJ, Spelsberg TC. TGFbeta inducible early gene-1 directly binds to, and represses, the OPG promoter in osteoblasts. *Biochem Biophys Res Commun*. 2010; 392:72–76. [PubMed: 20059964]
35. Venuprasad K, Huang H, Harada Y, Elly C, Subramaniam M, Spelsberg T, Su J, Liu YC. The E3 ubiquitin ligase Itch regulates expression of transcription factor Foxp3 and airway inflammation by enhancing the function of transcription factor TIEG1. *Nat Immunol*. 2008; 9:245–253. [PubMed: 18278048]
36. Cao Z, Wara AK, Icli B, Sun X, Packard RR, Esen F, Stapleton CJ, Subramaniam M, Kretschmer K, Apostolou I, von Boehmer H, Hansson GK, Spelsberg TC, Libby P, Feinberg MW. Kruppel-like factor KLF10 targets transforming growth factor-beta1 to regulate CD4(+)CD25(-) T cells and T regulatory cells. *J Biol Chem*. 2009; 284:24914–24924. [PubMed: 19602726]
37. Guillaumond F, Grechez-Cassiau A, Subramaniam M, Brangolo S, Peteri-Brunback B, Staels B, Fievet C, Spelsberg TC, Delaunay F, Teboul M. Kruppel-like factor KLF10 is a link between the circadian clock and metabolism in liver. *Mol Cell Biol*. 2010; 30:3059–3070. [PubMed: 20385766]
38. Talantov D, Mazumder A, Yu JX, Briggs T, Jiang Y, Backus J, Atkins D, Wang Y. Novel genes associated with malignant melanoma but not benign melanocytic lesions. *Clin Cancer Res*. 2005; 11:7234–7242. [PubMed: 16243793]

Fig. 1. Indicated 6Myc-tagged JMJD proteins were coexpressed with Flag-tagged TIEG1 in 293T cells. After anti-Myc immunoprecipitation, coprecipitated TIEG1 was revealed by anti-Flag Western blotting (top panel). The middle panel shows that comparable amounts of Flag-tagged TIEG1 were expressed, and the bottom panel indicates the expression levels of the 14 different JMJD proteins.

Fig. 2. Interaction of TIEG1 with JARID1B. (A) Coimmunoprecipitation of JARID1B with TIEG1. Flag-tagged JARID1B was expressed with 6Myc-TIEG1 or vector control in 293T cells. (B) Determination of interaction domains in TIEG1. Indicated Flag-tagged TIEG1 amino acids were coexpressed with 6Myc-JARID1B. After anti-Flag immunoprecipitation, coprecipitated JARID1B was detected by anti-Myc Western blotting. The bottom two panels show input levels of 6Myc-JARID1B or Flag-TIEG1 truncations. R1, 2, 3: repression domains. (C) TIEG1 binding domains in JARID1B. Binding of 6Myc-TIEG1 to indicated GST-JARID1B fusion proteins was assessed. The Coomassie stained gel at the bottom shows that comparable amounts of GST-JARID1B fusion proteins (marked by asterisks) were employed.

Fig. 3.

(A) Repression of the Smad7 promoter by TIEG1. Increasing amounts of TIEG1 expression plasmid were cotransfected with a Smad7 or CMV luciferase reporter gene. Luciferase activities compared to vector control are depicted. (B) Similar, the effect of increasing amounts of JARID1B expression plasmid on the Smad7 and CMV promoters was assessed. (C) Coexpression of TIEG1 (1 ng) with JARID1B (15 ng) expression plasmid shows cooperation in reducing Smad7 luciferase activity.

Fig. 4.

(A) MDA-MB-231 cells expressing control or shRNA directed against JARID1B. The top shows Western blots of JARID1B and actin as a loading control, whereas the bottom presents results from an RT-PCR experiment amplifying Smad7 mRNA or, as a control, GAPDH mRNA. (B) JARID1B and TIEG1 are both downregulated in melanomas (“M”; n=45) versus normal skin/benign skin nevi (“N”; n=7/18). Analysis was done with ONCOMINE (www.oncomine.org) utilizing data derived from published microarray results [38]. Shown are medians with respective 25–75 percentile intervals. Normalization was done by log2 transforming mRNA datasets, with the median set to zero and the standard deviation set to one. Significance was determined with Student’s *t* test.