Kaiso Directs the Transcriptional Corepressor MTG16 to the Kaiso Binding Site in Target Promoters

Caitlyn W. Barrett1,2, J. Joshua Smith6, Lauren C. Lu4, Nicholas Markham2, Kristy R. Stengel3, Sarah P. Short2, Baolin Zhang1,2, Aubrey A. Hunt3, Barbara M. Fingleton2,7, Robert H. Carnahan2, Michael E. Engel9, Xi Chen6,7, R. Daniel Beauchamp5,7, Keith T. Wilson1,2,7,8, Scott W. Hiebert3,7, Albert B. Reynolds2,7, Christopher S. Williams1,2,7,8

1 Division of Gastroenterology, Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America, 2 Department of Cancer Biology, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America, 3 Department of Biochemistry, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America, 4 School for Science and Math, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America, 5 Section of Surgical Sciences, Department of Surgery, Division of Surgical Oncology, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America, 6 Department of Biostatistics, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America, 7 Vanderbilt Ingram Cancer Center, Nashville, Tennessee, United States of America, 8 Veterans Affairs Tennessee Valley Health Care System, Nashville, Tennessee, United States of America, 9 Huntsman Cancer Institute, University of Utah, Salt Lake City, United States of America

Abstract

Myeloid translocation genes (MTGs) are transcriptional corepressors originally identified in acute myelogenous leukemia that have recently been linked to epithelial malignancy with non-synonymous mutations identified in both MTG8 and MTG16 in colon, breast, and lung carcinoma in addition to functioning as negative regulators of WNT and Notch signaling. A yeast two-hybrid approach was used to discover novel MTG binding partners. This screen identified the Zinc fingers, C2H2 and BTB domain containing (ZBTB) family members ZBTB4 and ZBTB38 as MTG16 interacting proteins. ZBTB4 is downregulated in breast cancer and modulates p53 responses. Because ZBTB33 (Kaiso), like MTG16, modulates Wnt signaling at the level of TCF4, and its deletion suppresses intestinal tumorigenesis in the Apcmin mouse, we determined that Kaiso also interacted with MTG16 to modulate transcription. The zinc finger domains of Kaiso as well as ZBTB4 and ZBTB38 bound MTG16 and the association with Kaiso was confirmed using co-immunoprecipitation. MTG family members were required to efficiently repress both a heterologous reporter construct containing Kaiso binding sites (4×KBS) and the known Kaiso target, Matrix metalloproteinase-7 (MMP-7/Matrilysin). Moreover, chromatin immunoprecipitation studies placed MTG16 in a complex occupying the Kaiso binding site on the MMP-7 promoter. The presence of MTG16 in this complex, and its contributions to transcriptional repression both required Kaiso binding to its site on DNA, establishing MTG16-Kaiso binding as functionally relevant in Kaiso-dependent transcriptional repression. Examination of a large multi-stage CRC expression array dataset revealed patterns of Kaiso, MTG16, and MMP-7 expression supporting the hypothesis that loss of either Kaiso or MTG16 can de-regulate a target promoter such as that of MMP-7. These findings provide new insights into the mechanisms of transcriptional control by ZBTB family members and broaden the scope of co-repressor functions for the MTG family, suggesting coordinate regulation of transcription by Kaiso/MTG complexes in cancer.

Citation: Barrett CW, Smith JJ, Lu LC, Markham N, Stengel KR, et al. (2012) Kaiso Directs the Transcriptional Corepressor MTG16 to the Kaiso Binding Site in Target Promoters. PLoS ONE 7(12): e51205. doi:10.1371/journal.pone.0051205

Editor: Reshma Taneja, National University of Singapore, Singapore

Received July 31, 2012; Accepted October 30, 2012; Published December 12, 2012

This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Funding: This work was funded by National Center for Research Resources, Grant UL1 RR024975-01, and is now at the National Center for Advancing Translational Sciences, Grant 2 UL1 TR000445-06, the Cellular and Biochemical and Molecular Sciences Training Program (NIH T32 GM08554), an NCI sponsored fellowship (CWB 1F31CA167920), and NIDDK K-08 (CSW 5K08DK080221). Additional funding from the Veterans Administration (CSW & KTW) and the American Cancer Society ACS-RSG 116552 (CSW). Core Services performed through Vanderbilt University Medical Center’s Digestive Disease Research Center supported by NIH grant P30DK058404 (RMP) and the Vanderbilt Ingram Cancer Center shared resources P30CA068485. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: christopher.williams@vanderbilt.edu

Introduction

The myeloid translocation gene proteins (MTGs) are transcriptional corepressors, lacking both enzymatic and DNA-binding activities, that act as scaffolding proteins upon which other transcriptional corepressors (mSin3a, N-CoR, SMRT), histone deacetylases (HDACs), and transcription factors assemble [1,2]. Their modular nature permits contributions to multiple promoter-specific repressor complexes, making them master regulators of gene expression. MTG8 and MTG16 (CBFA2T3) were originally identified as targets of chromosomal translocations in acute myeloid leukemia [1,2], suggesting an important role in cellular growth and development. MTG family proteins have since been implicated in the development of colon and breast cancers as well [3,4,5,6]. Because MTG repressive capabilities are dictated via protein-protein interactions with transcription factors and other co-repressors it is imperative to identify MTG-interacting proteins...
and understand their functions and specificities in the context of tumorogenesis.

Zinc fingers, C2H2 and BTB domain containing (ZBTB) family members bind methylated or unmethylated DNA in a sequence-specific manner and repress target genes [7]. A ZBTB family member that has recently been studied in detail for its ability to modulate colorectal cancer, Kaiso (ZBTB33), utilizes all three zinc fingers for high affinity binding to DNA targets [8]. All ZBTB family members likely share this method of DNA binding, as homology exists within their zinc finger motifs. Kaiso, like MTGs, forms repression complexes that include N-CoR and HDACs [9] and plays a pivotal role in the repression of tumor suppression genes such as CDKN2A in colon cancer cell lines [10]. Kaiso also represses matrix metalloproteinase gene MMP-7 expression [11] in addition to canonical and non-canonical Wnt targets [12,13,14]. Moreover, Kaiso expression is a prognostic indicator in non-small cell lung cancer [14] and its deletion suppresses intestinal tumorogenesis in mice [15]. ZBTB4 also modulates the cellular responses to p53 activation [16] and is downregulated in breast cancer [17]. Collectively, these data suggest ZBTB proteins, and Kaiso specifically, have multidimensional roles in cancer development.

Like the ZBTB family of transcription factors, the MTG family of transcriptional corepressors have disparate roles in cancer development. For example, MTGs associate with TCF4 to repress Wnt signaling [18] and mutations in both MTG8 and MTG16 were found in colon and breast cancer [4,5]. Mtgr1 is required for tumorigenesis in a murine model of inflammatory carcinogenesis [6], and MTG16 has been identified as a putative tumor suppressor in human breast cancer [3]. Given that ZBTB16 (also known as PLZF) and BCL6 (ZBTB27) associate with MTG8 [19,20] and that Kaiso and MTG16 have similar influences in cancer development, we tested for a wider structure-function relationship between the ZBTB and MTG families, which could provide insights into their roles in tumorogenesis.

Here, we identified the association of Kaiso, ZBTB4, and ZBTB38 with MTG16 in yeast two-hybrid assays, which suggests a direct physical interaction between these factors. The Kaiso-MTG16 complex specifically binds to Kaiso’s established binding site (KBS) on DNA and enhances repression of a KBS-containing reporter and the Kaiso target, MMP-7 promoter. However, MTG family members did not influence methylation-based repression by Kaiso. These data suggest that Kaiso-MTG16 dependent transcriptional repression requires the interaction of this complex on the KBS. Moreover, the impact of MTG16 on repression of Kaiso target promoters depends on Kaiso DNA binding. Analysis of publicly available ChIP-seq datasets showed that MTG family members bind Kaiso-targeted promoters over 70% of the time, implicating this interaction in the regulation of over 100 genes. Lastly we examined a large multi-stage CRC expression array implicating this interaction in the regulation of over 100 genes.

Materials and Methods

Yeast Two-Hybrid

ZBTB4 and ZBTB38 prey and MTG16 bait plasmids were obtained from Hybrigenics. pDONR201-Kaiso (clone ID: HsCD00082434, The ORFeome Collaboration) and pDONR221-MTG16 (clone ID: HsCD00079915, HIP) plasmids were obtained from Harvard PlasmID and pGAD-GW and pGBT9-GW vectors were provided by ABR. Kaiso and MTG16 were inserted into the pGAD-GW and pGBT9-GW plasmids using the Gateway Cloning procedure [21]. In short, 150 ng of entry vector (Kaiso or MTG16) was mixed with 150 ng of destination vector (pGAD or pGBT9) and the volume was brought to 8 µl with TE. 2 µl of LR Clonase II enzyme mix (Invitrogen) was added and the reaction was incubated for 1 hour at 25°C, 1 µl Proteinase K was then added to each sample and incubated at 37°C for 10 minutes. Kaiso was then truncated to include amino acids 298–573 by Agilent Quickchange II site-directed mutagenesis (Agilent) according to the manufacturer’s protocol. The MTG16 constructs shown in Figure 1 were also developed using the Quickchange II site-directed mutagenesis kit.

For the yeast two-hybrid, yeast at OD600 of 0.6 were centrifuged at 3000 rpm for 5 minutes and resuspended in 10 ml of LiAc-TE, centrifuged and resuspended in 1 ml of LiAc-TE. Transformations were set up using 50 µg of carrier DNA (sheared salmon sperm DNA, Ambion), 1 µg of DNA for each construct (pGAD and pGBT9) and 100 µl of competent yeast. 0.7 ml of sterile 40%PEG-LiAc-TE was added and yeast were allowed to recover at 30°C for 30 minutes and then heat shocked at 42°C for 15 minutes. Cells were then washed with sterile TE and the reactions were plated on double-dropout selective media plates (-leu, -trp) and grown at 30°C for three days.

Colonies were then plated on either triple- (for the Hybrigenics system) or quadruple-dropout (for the pGAD/pGBT9 system, -leu, -try, -his, -ala) plates. Empty vector bait was used as a negative control.

Immunofluorescence and Colocalization

K562 human leukemia cells (ATCC CCL-243) were plated on poly-l-lysine (Sigma-Aldrich) coated cover slips and allowed to adhere for 30 minutes at room temperature. Cells were fixed with ice-cold 2% paraformaldehyde, pH 7.4 for 10 minutes. After three washes, cells were blocked with 1% BSA and then stained with MTG16 antibody (1:1000 333, supplied by MEE) and a polyclonal Kaiso antibody (1:1000 supplied by ABR) overnight at 4°C. Cells were then washed then stained with anti-rabbit-Gy3 and anti-mouse-FTTC for 1 hour at room temperature and mounted using ProLong Gold antifade mounting medium with DAPI (Invitrogen). Slides were visualized using Delavision software (Stress Photonics, Madison, WI) supplied by the Epithelial Biology Core (Vanderbilt).

Cell Culture

Human colon cancer cell lines HCT116 (ATCC CCL-247) and HT29 (ATCC HTB-38) were maintained in McCoy’s 5A media supplemented with fetal bovine serum (FBS) and pen/strep (P/S). HCT116 cells were transfected with Lipofectamine LTX and Plus reagent (Invitrogen) and HT29 cells were transfected with Fugene HD (Promega) according to manufacturer’s protocol. K562 cells were maintained in DMEM supplemented with FBS and P/S.

Coimmunoprecipitation

HCT116 cells were transfected with Kaiso and each of the Gal or Myc-tagged MTG family members (provided by Mike Engel) using Lipofectamine LTX and Plus reagent (Invitrogen) according to manufacturer’s protocol. 48 hours post-transfection, cells were lysed with coimmunoprecipitation buffer (0.5% Triton-X 100, 0.1% DOC, 0.1% SDS, and protease inhibitors in PBS), clarified by centrifugation, and pre-cleared with protein A/G agarose beads for 1 hour at 4°C. 2 µg of either anti-Gal (Santa-Cruz, sc-510), anti-Myc (Santa-Cruz, sc-40) or anti-igG (Cell Signaling, G3A1) control antibodies were then added to pre-cleared lysate
and incubated overnight at 4°C with rotation. Following removal of input, 40 μl protein A/G agarose beads were added to each sample and incubated at 4°C for 1 hour with rotation. Western blotting for Kaiso (polyclonal Kaiso antibody supplied by ABR, 1:1000) was then performed.

**Kaiso Knockdown**

HEK293T packaging cells (ATCC CRL-11268) were transfected with Mission shRNA constructs (Sigma-Aldrich) specific for human Kaiso (clone ID: NM_006777.3-2600s1c1 and NM_006777.3-358s1c1) as well as the PAX2 and MDG2 plasmids using Superfect Transfection Reagent (Qiagen) according to the manufacturer’s protocol. 24 hours post-transfection, media was removed from the HEK293T cells and passed through a 0.2 μm filter. 4 μg/ml Polybrene (Millipore) was added to the filtered media. Growth medium was removed from HCT116 cells and replaced by infection medium. After 6 hours, the infection medium was replaced with normal growth medium and the infection process was repeated 24 hours later. Cells were selected

---

**Figure 1. ZBTB family members interact with MTG16.** A. ZBTB family member alignment based on homologous zinc fingers and identification of amino acids used in yeast-two hybrid experiments (yeast two-hybrid clones). B. Yeast-two hybrid assay for interactions between ZBTB family members and MTG16. C. Mapping of the Kaiso binding site on MTG16. Yeast two-hybrid assays were performed in triplicate. doi:10.1371/journal.pone.0051205.g001
for knockdown using 5 μg/ml Puromycin (Invitrogen) over a 3-day period. Knockdown was analyzed by the delta-delta-CT method following RT-PCR using Kaiso-specific Taqman probes (Invitrogen) and by Western blotting using the polyclonal Kaiso antibody (1:1000, supplied by ABR).

Luciferase Reporter Assays

Kaiso and MTG family members were transiently expressed in HCT116 cells along with either the Kaiso binding site (4×KBS) reporter, which contains four consensus Kaiso binding sites and a luciferase reporter gene (gift from Juliet Daniel, McMaster University, Canada), or the mutant Kaiso binding site promoter which contains mutant forms of the KBS and a luciferase reporter gene (PGL3 control 4×KBSMT, gift from Juliet Daniel). A constitutive reporter plasmid expressing Renilla luciferase (pGL4-TK hRluc) was included to normalize data generated from the Kaiso reporter constructs. Cell lysates were subjected to a dual-luciferase assay and presented as adjusted RLU's. For human MMP-7 (HMAT, gift from BMF) assays, Myc-tagged delta-MTG16 constructs (ME, Figure 1C) were co-transfected with activators PEA3, LEF1, C-JUN, and delta-β-Catenin and the human MMP-7 promoter fused to the luciferase reporter gene (HMAT-2.3). Activation by enforced expression of PEA3, LEF1, C-JUN, and delta-β-catenin was required to see repression by MTG family members because HCT116 cells express low levels of MMP-7 endogenously. Mbd2<sup>−/−</sup> cells (gift from Pierre-Antoine Defossez) [22] were maintained in Advanced MEM supplemented with BCS and P/S and transfected with Superfect (Qiagen), according to manufacturer’s protocol. For methylation-dependent studies, 5 μg of pSV40Luc was methylated upon mixture of 1.5 μl SAM, 30 μl 10× NEB2, 1 μl SsI methylase (New England Biolabs) and water to 300 μl. The mixture was incubated at 37°C for 2 hours followed by addition of 1.5 μl SAM and continued incubation for 1 hour. DNA was then purified using the Qiagen PCR purification kit (Qiagen). Mbd2<sup>−/−</sup> cells (gift from Pierre-Antoine Defossez) were transfected with MTG family members and either methylated or unmethylated promoter according to previously published specifications [22]. HMAT and KBS assays were performed as outlined in the Materials and Methods section in the main paper.

MMP-7 Repression

HT29 cells were transfected with expression plasmids for Kaiso, an MTG family member, or a combination of the two. RNA was isolated using the Qiagen RNA Isolation Kit and cDNA was generated from 1 μg of total RNA using the iScript cDNA Synthesis Kit (BioRad). Taqman qPCR was performed using MMP-7 specific probes (Invitrogen), and expression was normalized to GAPDH (Invitrogen). Analysis was performed using the delta-delta Ct method. Transfected cells were also treated with Protein transport inhibitor containing Brefeldin A (BD GolgiPlug) for 3 hours before lysis was collected using RIPA buffer. Lysates were run on Western blots, probed with α-MMP-7 (Santa Cruz 1:1000) and α-β-actin (Sigma-Aldrich, 1:5000) as a loading control and developed using the Odyssey system.

Chromatin Immunoprecipitation

HCT116 cells were fixed using 1% formaldehyde for 20 minutes at room temperature. Cells were then lysed and chromatin was sheared by sonication (4 watts, 10 seconds on, 1 minute off, on ice repeated ten times). Lysate was preclarred with 20 μl protein A/G agarose beads (Santa Cruz, sc-2003) and beads were blocked with 2 ug sheared salmon sperm DNA (Ambion). Lysate was immunoprecipitated with either IgG (Cell Signaling, G3A1), polyclonal MTG16 (333, provided by MEE) or monoclonal MTG16 (2DH, provided by SWH) overnight at 4°C with rotation. Complexes were eluted and crosslinks reversed with NaCl at 65 degrees for 5 hours and DNA isolated using the Qiagen PCR purification kit. qRT-PCR was performed using Kaiso binding site-specific primers [11]. Analysis was performed using the delta-delta Ct method.

Statistical Methods

Luciferase, ChIP, and expression analyses were analyzed using one-way ANOVA and a Newman-Keuls post-test in Graphpad Prism 5.0c, unless otherwise indicated.

Microarray Analysis and Statistics

Microarray data were normalized using the Robust MultiChip Averaging (RMA) algorithm as implemented in the Bioconductor package Affy as previously described [23,24,25]. Wilcoxon Rank sum test was used to determine significance for the normal, adenoma and colorectal cancer comparisons. The gene expression data Spearman correlation coefficient was used to assess the association between Kaiso and MTG16 (probes 214631_at and 208056_s_at respectively). We next compared the differential expression between normal samples and carcinoma samples for genes MTG16 and MMP-7 using a linear model with an interaction term. More specifically, our model included gene expression as the outcome variable, main effects group (normal, carcinoma) and gene (e.g., Kaiso, MMP-7) as well as the group x gene interaction term. The significance of interaction term indicates that differential gene expression for normal vs. carcinoma is significantly different for the two genes. This test procedure was similarly applied to genes Kaiso versus MMP-7.

Ethics Statement

The protocols and procedures for this study were approved by the Institutional Review Boards at University of Alabama-Birmingham Medical Center, Vanderbilt University Medical Center, Veterans Administration Hospital (Nashville, TN) and H. Lee Moffitt Cancer Center, and written informed consent was obtained from each subject per institutional protocol.

Results

ZBTB family members interact with MTG16

In order to understand MTG16-mediated intracellular signaling we used yeast-two hybrid screening to identify MTG16 binding partners. This approach identified two members of a family of
zinc-finger transcription factors that repress methylated and non-methylated DNA: ZBTB4 and ZBTB38. Another member of this family, Kaiso (ZBTB33), is of particular interest because, like MTG16, it has been previously linked to Wnt signaling and tumorigenesis in the gut [10, 12, 15]. Kaiso was not identified in the initial yeast two-hybrid screen, likely due to limited expression in the murine brain library that was screened. However, the ZBTB4 and ZBTB38 clones that were identified consisted of the highly homologous zinc finger region shared by ZBTB family members (Fig. 1A, S1) and suggested that Kaiso would likely be a third target for MTG16 binding. Directed yeast two-hybrid validation experiments demonstrated that ZBTB4, ZBTB38, and Kaiso each interact with MTG16 via a highly conserved zinc-finger domain (Fig. 1B) which is also required for ZBTB16 (PLZF) MTG16 interaction [19], but not with ZBTB27 (BCL6) which demonstrates MTG16 binding within the fourth zinc finger motif [20](Fig. S1). Mapping of the MTG16 binding domain identified the nervy homology region 1 (NHR1) as the component of MTG16 that is required for binding to Kaiso as the three constructs lacking the NHR1 domain (∆N5, ∆N7, and ∆NHR1) failed to demonstrate an interaction in the yeast two-hybrid assay (Fig. 1C).

Using wild-type and selected mutant constructs for MTG16, we confirmed the Kaiso interaction in the HCT116 human colon cancer cell line which has endogenous Kaiso expression (Fig. 2A, r = 0.95 ± 0.04). We then mapped the binding interface to the N-terminus of MTG16 (Fig. 2B,C). We next used immunofluorescence to demonstrate endogenous co-localization of Kaiso and MTG16 in the K562 cells, a human leukemia cell line with high levels of expression of both proteins (Fig. 2D). Furthermore, in silico analysis of publicly available ChIP-seq data (UCSC Genome Browser, [26]) identified 101 promoters with intersecting Kaiso and MTG protein occupancy (Fig. S2A), providing further evidence that cooperativity between Kaiso and the MTG proteins may occur. Furthermore, three targets identified in the in silico ChIP-seq analysis, ATF-2, ATF-7, and MAPK14 demonstrate altered expression in response to MTG16 overexpression. ATF-2 and MAPK14 are repressed by MTG16 (Fig. S2B, P<0.0001 ATF-2 and P=0.04 MAPK14) while ATF-7 expression is higher in response to MTG16 overexpression (Fig. S2C, P<0.0001). Though initially surprising, this result still points to a Kaiso and MTG16 complex binding the ATF-7 promoter but suggests that either the proteins behave as activators, something Kaiso is capable of on this promoter, or that they are displacing another repressor complex that is a more potent repressor than the Kaiso-MTG16 complex. Collectively, these findings suggest a direct binding relationship between Kaiso and MTG16 with a shared impact in transcriptional control and tumorigenesis.

**MTG family members repress the MMP-7 promoter**

In mammalian cells, Kaiso recognizes both the consensus sequence TCCTGGCNA (Kaiso binding site, KBS) and methylated CpG dinucleotides [22, 27]. We used a luciferase-based Kaiso binding site reporter (4×KBS), as well as a mutant form that contains a C→A transversion that abolishes both Kaiso binding and Kaiso-mediated repression (4×KBSMT) [27] to explore cooperation between Kaiso and MTG family proteins in transcriptional repression (Fig. 3A). We determined that MTGs were capable of repressing the 4×KBS reporter construct (Fig. 3B) and that this repression was specific as it was disrupted when the 4×KBSMT reporter was used in lieu of the 4×KBS reporter (Fig. 3C). Additionally, Kaiso knockdown (Fig. 3D, left) resulted in a dose-dependent loss of 4×KBS repression by MTG16 (Fig. 3D, right). Finally, knockdown of both MTG16 and Kaiso resulted in decreased repression of the KBS in the HCT 116 cell line that expresses high levels of both proteins (Fig. S3A & B, P<0.0001).

Because we determined MTG family members are able to repress the sequence-specific Kaiso target, we also wanted to determine whether MTGs were able to repress the second common Kaiso target, methyl-CpG. We therefore assessed the ability of MTG family members to repress a methylated reporter plasmid [22]. In this case, we saw little repression of the methylated reporter by MTG family members until very high levels of MTG8 or MTG16 were used (Fig. 3E). This indicates that Kaiso dependent MTG repression specificity is directed towards genes containing the KBS as opposed to methyl-CpG, and suggests a model where MTG16 cooperates with Kaiso in the repression of KBS targets, but not to methylated CpG targets (Fig. 3F).

**MTG family members repress the MMP-7 promoter**

MMP-7 is an established tumor promoter in colon, prostate, pancreas, and lung cancers [28, 29, 30, 31]. It also contributes to tumor progression and metastasis [32]. Interestingly, two Kaiso binding sites are present in the human MMP-7 promoter (Fig. 4A) that enable Kaiso-mediated repression of MMP-7 expression [27]. Our data suggests that MTGs may cooperate with Kaiso to repress Kaiso targets. Therefore, we hypothesized that MTGs would repress the MMP-7 promoter. Titration of each MTG family member with the full-length human MMP-7 reporter (HMAT-2, [27]) in the Kaiso-expressing HCT116 cells resulted in dose-dependent repression (Fig. 4B). This repression was lost when an MTG16 construct lacking the NHR1 domain (loss of affinity mutant) was used, further supporting an interaction with Kaiso that is dependent on the NHR1 domain of MTG16 (Fig. 4C). Also, Kaiso knockdown resulted in loss of HMAT repression, suggesting that Kaiso represses MMP-7 via MTG16 recruitment (Fig. 3D & 4D). Moreover, knockdown of both MTG16 and Kaiso in the HCT116 cell line resulted in decreased repression of the HMAT reporter (Fig. S3A & C, P<0.0001). Taken together, these data demonstrate that MTG16 and Kaiso repress MMP-7 promoter activity.

The human MMP-7 promoter also contains two TCF binding sites (Fig. 5A). MTGs also associate with TCF4 and repress TCF4 targets [18]. Thus it was possible that in addition to repressing via an interaction with Kaiso at the KBS, MTG16 could be recruited by TCF4 to repress MMP-7 via TCF response elements. To address this issue of MTG16 specificity for Kaiso or TCF binding sites, we performed repression assays using heterologous transcriptional reporters composed of minimal MMP-7 promoter elements where TCF binding sites were mutated individually or in combination [33] (Fig. S4A). Mutation of either or both of these sites had no effect on MTG16-mediated repression of MMP-7 (Fig. S4B). These data indicate that MTG16-dependent MMP-7 repression occurs independent of TCF binding sites. In concert, these results suggest that MTG16 recruitment by Kaiso to the MMP-7 KBS is responsible for MTG16/Kaiso mediated MMP-7 repression.

Both MTG16 and Kaiso have been implicated in breast, colon, and lung carcinoma [3, 4, 5, 10, 34, 35]. Each can regulate Wnt signaling [12, 18], a pathway strongly associated with colon cancer development. Recently at least five MTG mutations have been identified in colon or breast cancer [4, 5], all are non-synonymous and predicted to influence MTG function. We therefore wanted to determine whether these mutations could influence Kaiso dependent repression of the 4×KBS and HMAT reporters. Overexpression of each MTG mutant resulted in no change in
repression of either the 4×KBS (Fig. S5A) or HMAT reporters (Fig. S5B), indicating that these mutations do not influence MTG dependent Kaiso repression.

**MTG family members repress endogenous MMP-7 expression**

Based upon the transcriptional control data described above, we predicted that MTG16 should occupy the promoters of Kaiso-regulated genes. To address this hypothesis, we used ChIP to probe for MTG16 occupancy of the −537 Kaiso binding site in the endogenous MMP-7 promoter (Fig. 5A). We observed 13.2 or 14.7 fold enrichment over IgG when either a polyclonal (333, pMTG) or monoclonal MTG16 (2D1, mMTG16) antibody was used (P<0.001, Fig. 5B). Importantly, Kaiso knockdown abrogated MTG16 binding to the MMP-7 promoter, showing that Kaiso is essential for MTG16 recruitment (Fig. 5C, D).

We next determined whether MTG16 was capable of regulating endogenous MMP-7 expression by analyzing MMP-7 mRNA and protein levels in the context of enforced expression of MTG family members. For this experiment, the colon cancer cell line HT29 was utilized. We chose HT29 cells because they express and secrete high levels of MMP-7 and express little MTG16 compared to other colon cancer cell lines, making them ideal for establishing repression of endogenous MMP-7 (Fig. S6A, B). Overexpression of MTG8, MTG16, and MTGR1 resulted in decreased MMP-7 both at the RNA and protein levels when compared to a vector control (fold-change mRNA expression: 0.60 S.E. 0.05 (MTG8), 0.59 S.E. 0.07 (MTG16), 0.71 S.E. 0.09 (MTGR1), P<0.01 for each, Fig. 5E), and this data was confirmed in a non-cancer cell line, Cos7 (Fig. S7). Considering that HT29 transfection efficiency in these experiments was 44.85%±6.553, the data likely under-represent the impact of MTGs on MMP-7 expression. Moreover, knockdown of Kaiso and MTG16 in HCT116 cells resulted in derepression of endogenous MMP-7 expression (Fig. S3D, P=0.04). These findings suggest that MTGs repress endogenous MMP-7 expression via interaction with Kaiso.
Figure 3. MTG family members repress the Kaiso binding site reporter (4×KBS).

A. Composition of the KBS reporter constructs. The 4×KBS reporter contains four consensus Kaiso binding site sequences followed by a luciferase gene. The mutant reporter (4×KBSMT) contains a C→A transversion in the Kaiso binding sites which abolishes Kaiso binding.

B. 4×KBS artificial promoter assays in HCT116 cells upon titration of MTG family members (0 ng, 200 ng, 400 ng, 800 ng). The graph shows the fold-change in luciferase activity relative to the control reporter, pGL4-TK hRLUC, after treatment with different concentrations of MTG family members.

C. Comparison of luciferase activity between the wild-type and mutant reporters in HCT116 cells treated with different concentrations of MTG family members.

D. Quantification of Kaiso mRNA levels in cells treated with sh-scrambled, sh-hKaiso1, and sh-hKaiso2. The graph shows the fold-change in Kaiso mRNA levels relative to β-actin.

E. Methylation status of the pSV40 reporter in cells treated with different concentrations of MTG family members.

F. Schematic representation of the MTG family members' interaction with the Kaiso binding site.
transfection of expression plasmids encoding MTG family members, MTG8, MTG16, and MTGR1. The error bars represent the standard error of the mean for 3 replicate experiments performed in triplicate. C. 4×KBS and 4×KBSMT artificial promoter assays in HCT116 cells after transfection of 500 ng of the indicated MTG family member. The error bars represent the standard error of the mean for 4 replicate experiments performed in triplicate. D. Kaiso knockdown by two independent Kaiso shRNA constructs compared to a scrambled shRNA control (left). 4×KBS artificial promoter assay after knockdown of Kaiso and the transfection of 500 ng of MTG16 (right). E. Methylated pSV40 artificial promoter assay in Mbd2−/− cells upon titration of MTG family members (0 ng, 200 ng, 400 ng, 800 ng). The graph shows the fold-change in luciferase activity relative to the control reporter, pGL4-TK hRLUC, after transfection of expression plasmids encoding MTG family members, MTG8, MTG16, and MTGR1. The error bars represent the standard error of the mean for 3 replicate experiments performed in triplicate. F. Model for Kaiso repression of Kaiso binding site (KBS) and methyl CpGs (mCpG) on target promoters. *P<0.05, **P<0.01, ***P<0.001.

doii:10.1371/journal.pone.0051205.g003

Figure 4. MTG family members depend on Kaiso to repress the human MMP-7 promoter. A. The composition of the full-length MMP-7 reporter (HMAT-2.3). The HMAT-2.3 reporter contains two Kaiso and two TCF binding sites followed by a luciferase gene. B. HMAT-2.3 artificial promoter assays in HCT116 cells upon titration of MTG family members (0 ng, 200 ng, 400 ng, 800 ng). The graph shows the fold-change in luciferase activity relative to the control reporter, pGL4-TK hRLUC, after transfection of expression plasmids encoding MTG family members, MTG8, MTG16, and MTGR1. The error bars represent the standard error of the mean for 3 replicate experiments performed in triplicate. C. HMAT-2.3 promoter assay utilizing MTG16 deletion constructs in which the indicated nervous homology region is removed. Error bars represent the standard deviation of triplicate samples. D. HMAT-2.3 artificial promoter assay after knockdown of Kaiso and the transfection of 500 ng of MTG16. Error bars represent the standard deviation of triplicate samples. *P<0.05, ***P<0.001.

doii:10.1371/journal.pone.0051205.g004
MMP-7 increased expression is associated with decoupling of the MTG16 and Kaiso regulatory axis

We next sought to compare the expression levels of Kaiso, MMP-7 and MTG16 in patients with colorectal cancer compared with normal adjacent colon tissues and adenomas. We observed a significant up-regulation of MMP-7 and Kaiso (Fig. 6A, B, \( P<0.001 \) for all stages) while MTG16 is significantly down-regulated for all stages of cancer compared with normal adjacent colon samples (Fig. 6C, \( P<0.001 \)). Given the in vitro data demonstrating MTG16 played a key role and was required for maximal repression of Kaiso-regulated KBS-containing promoters, we reasoned that Kaiso and MTG16 expression would be inversely related on a per
sample basis. We did in fact note a highly significant inverse correlation (Fig. 6D, Rho = -0.29, P<0.001). The data presented in this paper suggests a model by which both Kaiso and MTG16 are necessary for MMP-7 repression. This model likely applies to other Kaiso:MTG16 regulated KBS containing promoters.

Discussion

Kaiso and MTG16 share several functional similarities, including their abilities to repress Wnt signaling, bind co-repressors, and impact processes that influence tumorigenesis. Because of these similarities, we postulated that Kaiso and MTGs could form complexes and that Kaiso could target MTG repression functions to specific promoters. In testing this hypothesis we found that Kaiso and MTG16 interact and that this depends on the homologous zinc fingers in Kaiso and the NHR1 domain in MTG16. Furthermore, when MTG family members are overexpressed, they enhance repression of a KBS reporter. Studies with both heterologous KBS reporters and Kaiso knockdown showed that MTG-dependent transcriptional repression on Kaiso targets, such as the MMP-7 promoter, is Kaiso-dependent. ChIP experiments revealed that the Kaiso-MTG interaction occurs endogenously at the KBS in the MMP-7 promoter, and reduced MMP-7 expression at both the RNA and protein level show the interaction to be functionally significant. Thus, we have linked two families of transcriptional repressors, both previously implicated in oncogenesis. Understanding the nature of this relationship is important in understanding transcriptional regulation by these complexes in normal biology and how this is perturbed in tumorigenesis.

Figure 6. MTG16 and Kaiso are negatively correlated in human CRC patient samples and MMP-7 is up-regulated. A) Kaiso expression is significantly up-regulated in adenomas and colorectal cancer patients compared with normal adjacent colon tissues (P<0.001, for all comparisons). B) MMP-7 expression is significantly up-regulated in adenoma and colorectal cancer patients for each stage (P<0.001 for all comparisons). C) MTG16 expression is significantly down-regulated in colorectal cancers versus normal adjacent samples (P<0.001; *for all comparisons except adenoma). Wilcoxon Rank Sum test was used to determine significance for A–C. D) Normalized expression of both MTG16 and Kaiso probes (see Methods) were compared in human colorectal cancer patients. The graph demonstrates a significant inverse correlation for MTG16 versus Kaiso (rho = -0.29, P<0.001; inverse correlation depicted by red line, Spearman correlation coefficient).

doi:10.1371/journal.pone.0051205.g006
Kaiso is a dual-specificity repressor that can recognize both a consensus sequence (KBS, TCCTGGCNA) and methyl-CpG dinucleotides [27]. Interestingly, though MTG16 is important for repression of KBS reporters, it does not repress a methylated reporter, suggesting that the Kaiso-MTG16 interaction is specific for promoters that contain the Kaiso binding site. Kaiso has been identified as the factor that recruits the N-CoR complex to CpG-rich sequences in a methylation-dependent manner [36], our study demonstrates that there is a converse activity with interaction with MTG16, as it is a Kaiso cofactor that is dependent solely on Kaiso binding to the canonical KBS. This suggests that Kaiso might differentiate between target repression based on the availability of cofactors, making these cofactors key in understanding the regulation of Kaiso targets, be they methylated or the canonical consensus sequence. We think that this is biologically relevant as we noted a strong inverse correlation between MTG16 and Kaiso levels in a large human CRC dataset. This could have important implications for the regulation of Kaiso:MTG16 targets and may influence the bimodal effect of Kaiso on carcinogenesis [10,12,15,35].

This interaction is also of great importance as our data directly implicates MTG16:Kaiso complexes in the regulation of MMP-7 expression. MMP-7 plays a significant role in all stages of tumor progression and is a key player in a wide variety of cancers including, but not limited to, colon, prostate, lung and ovarian [29,37,38,39,40]. Indeed, metalloproteinases are among the most common targets for anti-cancer drug development [41] so understanding their regulation will give insight into mechanisms by which their expression might be altered. We have determined that MTG16 binds Kaiso at the MMP-7 promoter and recruits transcriptional corepressors to attenuate MMP-7 expression. Furthermore, both MTG16 and Kaiso demonstrate coordinate patterns of expression when compared with MMP-7 in human CRC samples vs. normal adjacent colon samples. Decreased repression upon loss of either protein within this complex could contribute to tumorigenesis.

In order to better understand the scope of the Kaiso-MTG interaction, we analyzed public-domain ChIP-seq datasets (UCSC Genome Browser, [26]) to determine the overlap of targets bound by Kaiso and the MTG family members MTG16 and MTGR1 (Fig. S2A). Several targets relevant to cancer pathogenesis were revealed using this methodology. For example, Kaiso and MTG16 bind the Activating Transcription Factor (ATF)-2 promoter, while Kaiso and MTGR1 bind the promoter for ATF7. The ATF family is composed of transcription factors that are involved in cellular stress response [42]. Aside from being activated by the MAPK pathway in response to stress, ATFs 2 and 7 are also involved in oncogenesis, as they are able to dimerize with c-Jun to influence Jun-dependent survival, apoptosis, and cell cycle progression [43,44]. C-Jun pathway regulation is also seen with a third target of MTG-Kaiso complexes, MMP-7 (Fig. S2B). Several targets relevant to cancer pathogenesis were revealed using this methodology. For example, Kaiso and MTG16 bind the Activating Transcription Factor (ATF)-2 promoter, while Kaiso and MTGR1 bind the promoter for ATF7. The ATF family is composed of transcription factors that are involved in cellular stress response [42]. Aside from being activated by the MAPK pathway in response to stress, ATFs 2 and 7 are also involved in oncogenesis, as they are able to dimerize with c-Jun to influence Jun-dependent survival, apoptosis, and cell cycle progression [43,44]. C-Jun pathway regulation is also seen with a third target of MTG-Kaiso complexes, MMP-7 (Fig. S2B). Several targets relevant to cancer pathogenesis were revealed using this methodology. For example, Kaiso and MTG16 bind the Activating Transcription Factor (ATF)-2 promoter, while Kaiso and MTGR1 bind the promoter for ATF7. The ATF family is composed of transcription factors that are involved in cellular stress response [42]. Aside from being activated by the MAPK pathway in response to stress, ATFs 2 and 7 are also involved in oncogenesis, as they are able to dimerize with c-Jun to influence Jun-dependent survival, apoptosis, and cell cycle progression [43,44]. C-Jun pathway regulation is also seen with a third target of MTG-Kaiso complexes, MMP-7 (Fig. S2B).

In summary, we identified an association between Kaiso and MTG family members. Because Kaiso is the prototypic ZBTB family member, we focused on understanding the contribution of MTG16 to Kaiso mediated repression. We determined that the Kaiso-MTG16 complex specifically binds to the KBS and represses the Kaiso target, MMP-7. We examined a large multi-stage CRC expression array dataset and discovered an inverse relationship between Kaiso and MTG16 expression and consistently elevated MMP-7 expression at all stages of tumorigenesis supporting the hypothesis that loss of either Kaiso or MTG16 deregulates MMP-7 expression. Analysis of publicly available ChIP-seq datasets showed that MTG family members bind Kaiso-targeted promoters over 70% of the time, implicating this interaction in the regulation of over 100 genes, many of which are involved in cell cycle control and survival programs.

In conclusion, we report that Kaiso and MTGs interact in inhibitory complexes and identify a subset of target genes which are important in oncogenesis. Additional targets identified in the ChIP-seq screen include stress and oxidative damage response proteins potentially important in inflammatory carcinogenesis. Future experiments should be directed towards understanding the role of Kaiso and MTG16 in sporadic and inflammatory colon carcinogenesis.

Supporting Information

Figure S1 ZBTB family members interact with Kaiso through their homologous zinc finger domains. Uniprot alignment between the five ZBTB family members known to bind MTG16 indicates homology within the zinc fingers (highlighted in purple) and BTB (highlighted in green) domains of each protein (white indicates 0% homology and dark blue indicates 100% homology). Yeast-two hybrid assay indicates binding of Kaiso (orange underline) ZBTB4 (black underline) and ZBTB38 (red underline) to MTG16 within the homologous zinc finger region in a similar manner to ZBTB16 (green underline).

Figure S2 Kaiso shares repression targets with MTG16 and MTGR1. A. The UCSC genome browser ChIP-seq data was used to identify Kaiso targets. Chip-seq data developed by Soler et al., 2011 [26] was used to identify MTG16 and MTGR1 targets that overlapped with Kaiso binding sites. B. ATF-2, MAPK14, and C. ATF-7 mRNA expression upon overexpression of the MTG16. The graph shows the fold-change (ΔΔCt) of mRNA compared to an empty vector control. Error bars represent the standard error for three replicate experiments performed in triplicate. *P<0.05, **P<0.001.

Figure S3 Repression of 4×KBS, HMAT, and MMP-7 is decreased with knockdown of MTG16 and Kaiso in HCT116 cells. A. Kaiso or MTG16 mRNA expression after knockdown of Kaiso (sh-Kaiso) and MTG16 (si-MTG16) in HCT116 cells. The graph shows the fold-change (ΔΔCt) of mRNA compared to a scrambled control (sh-scrambled and si-scrambled). B. 4×KBS and C. HMAT reporter activity after knockdown of both Kaiso and MTG16. D. MMP-7 expression in response to knockdown of Kaiso and MTG16. Error bars represent the standard error for three replicate experiments performed in triplicate. **P<0.01, ***P<0.001.

Figure S4 MTG16 does not repress the MMP-7 reporter via TCF binding sites. A. The composition of the truncated MMP-7 reporter (HMAT-296) and TCF mutant reporters (HMAT-194, HMAT-109, and HMAT-2). B. HMAT artificial promoter assays in HCT116 cells. HCT116 cells were transfected
with either 500 ng of MTG16 or 500 ng of Empty vector as a control. The graph shows the fold-change in luciferase activity relative to the standard pGL4-1K hRLUC after transfection of expression plasmids. The error bars represent the standard error of four replicate experiments performed in triplicate. *P<0.05, **P<0.01.

Figure S5 Established MTG8 and MTG16 colorectal cancer mutations do not alter repression of Kaiso target promoters. A. 4×KBS artificial promoter assays in HCT116 cells. The graph shows the fold-change in luciferase activity relative to the standard pGL4-1K hRLUC after transfection of expression plasmids encoding the indicated MTG8 mutant constructs. B. HMAT-2.3 artificial promoter assays in HCT116 cells. The graph shows the fold-change in luciferase activity relative to the standard pGL4-1K hRLUC after transfection of expression plasmids encoding the indicated MTG8 mutant constructs. The error bars represent the standard error of three replicate experiments performed in triplicate. *P<0.05, **P<0.01.

Figure S6 HT29 cells express higher levels of MMP-7 and lower levels of MTG16 than other colon cancer cell lines. A. MTG16, B. MMP-7, and C. Kaiso mRNA expression in colon cancer cell lines. The graph shows the fold-change (ΔΔCt) of mRNA. Error bars represent the standard error for three replicate experiments performed in triplicate.

Acknowledgments

We would like to thank members of the Williams lab and Paul Barrett for helpful comments regarding this manuscript and Joseph Roland for technical assistance with the Delivision System. We thank Howard Crawford for the matrilisin reporter constructs and Julieta Daniel for her kind gift of the 4×KBS vectors. Finally, we would like to thank Brian Hendrich and Pierre-Antoine Defossez for providing the Mbd2−/− cells and technical advice regarding their use in methylation studies.

Author Contributions

Conceived and designed the experiments: CBW CSW ABR SWH. Performed the experiments: CWB LCL NM KRS SPS. Analyzed the data: CBW JJS. Contributed reagents/materials/analysis tools: CBW JJS NM KRS BZ AHR BMF RHC MEE XCG RDB. Wrote the paper: CBW JJS MEE KTW SWH ABR CSW.

References

1. Davis JN, McGhee L, Meyers S (2003) The ETO (MTG8) gene family. Gene 303: 1–10.
2. Lutterbach B, Westendorf JJ, Linggi B, Patten A, Moinia M, et al. (1998) ETO, a target of t(8;21) in acute leukemia, interacts with the N-CoR and mSin3 corepressors. Mol Cell Biol 18: 7176–7184.
3. Kochetkova M, McKenzie OL, Bais AJ, Martin JM, Secker GA, et al. (2002) CBP/3AT3 (MTG16) is a putative breast tumor suppressor gene from the breast cancer loss of heterozygosity region at 16p24.3. Cancer Res 62: 4999–4904.
4. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, et al. (2006) The consensus coding sequences of human breast and colorectal cancers. Science 314: 268–274.
5. Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, et al. (2007) The genomic landscapes of human breast and colorectal cancers. Science 318: 1108–1113.
6. Barrett CW, Fingleton B, Williams A, Ning W, Fischer MA, et al. MTGR1 is required for tumorigenesis in the murine AOM/DSS colitis-associated carcinoma model. Cancer Res 71: 1302–1312.
7. Sasai N, Nakao M, Defossez PA Sequence-specific recognition of methylated DNA by human zinc-finger proteins. Nucleic Acids Res 38: 5015–5022.
8. Bethany A. Buck-Koehnert MAM-YH, H June, Pyke-H Wright (2012) Kaiso uses all three zinc fingers and adjacent sequence motifs for high affinity binding to sequence-specific and methyl-CpG DNA targets. FEBS J 586: 734–739.
9. Filan GJ, Zhuo R, Salo A, Yamada D, Prokhorchouk E, et al. (2006) A family of human zinc finger proteins that bind methylated DNA and repress transcription. Mol Cell 26: 169–181.
10. Lopes EC, Vallis E, Figueiroa ME, Mazur A, Meng FG, et al. (2008) Kaiso contributes to DNA methylation-dependent silencing of tumor suppressor genes in colon cancer cell lines. Cancer Res 68: 7258–7263.
11. Spring CM, Kelly KF, O’Keeley I, Graham M, Crawford HC, et al. (2005) The c-Jun-dependent repression of the beta-catenin/TCF target gene matrilisin. Exp Cell Res 303: 253–263.
12. Puckett Cell, Lyons JP, Lin J, Nguyen TT, et al. (2005) Kaiso/p120-catenin and TCF/beta-catenin complexes coordinately regulate canonical Wnt target genes. Dev Cell 8: 843–854.
13. Del Valle-Berry B, Casagolda D, Luglade E, Vallis G, Codina M, et al. Wnt controls the transcriptional activity of Kaiso through C/EBPalpha-dependent phosphorylation of p120-catenin. J Cell Sci 124: 2298–2309.
14. Dai SD, Wang Y, Zhang JY, Zhang D, Zhang RX, et al. Uptregulation of delta-catenin is associated with poor prognosis and enhances transcriptional activity through Kaiso in non-small-cell lung cancer. Cancer Sci 102: 93–103.
15. Prokhorchouk A, Sansom O, Sellidge J, Caballero IM, Salo A, et al. (2006) Kaiso-deficient mice show resistance to intestinal cancer. Mol Cell Biol 26: 199–208.
16. Weber A, Marquardt J, Elci D, Forster N, Starek S, et al. (2008) Zbtb16 represses transcription of P21CIP1 and controls the cellular response to p53 activation. EMBO J 27: 1563–1574.
17. Kim K, Chashalopoul G, Lee SO, Yamada D, Sastre-Garau X, et al. Identification of oncogenic microRNA-17-92/ZBTB16/specification protein axis in breast cancer. Oncogene 31: 1034–1044.
18. Moore AC, Annan JM, Williams CS, Talinci E, Farmer TE, et al. (2008) Myeloid translocation gene family members associate with T-cell factors (TCFs) and influence TCF-dependent transcription. Mol Cell Biol 28: 977–987.
19. Melnick AM, Westendorf JJ, Polinger A, Carlile GW, Arai S, et al. (2000) The ETO protein disrupted in t(8;21)-associated acute myeloid leukemia is a co-repressor for the promyelocytic leukemia zinc finger protein. Mol Cell Biol 20: 3073–2086.
20. Chevallier N, Corcoran CM, Leunon C, Hynek E, Chadburn A, et al. (2004) ETO protein of t(8;21) AML is a corepressor for Bcl-1 B-cell lymphoma oncoprotein. Blood 103: 1454–1463.
21. Hartley JL, Temple G F, Brach MA. (2000) DNA Cloning Using In Vitro Site- Specific Recombination. Genome Res 10: 1798–1795.
22. Prokhorchouk A, Hendrich B, Jorgensen H, Ruozov A, Wilm A, et al. (2001) The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. Genes Dev 15: 1613–1618.
23. Smith JF, Deeke NG, Wu F, Merchant NB, Zhang B, et al. Experimentally derived metastasis gene expression profile predicts recurrence and death in patients with colon cancer. Gastroenterology 138: 958–968.
24. Williams CS, Zhang B, Smith JF, Jayagopal A, Barrett CW, et al. BVES regulates EMT in human colorectal and colon cancer cells and is silenced via promoter methylation in human colorectal carcinoma. J Clin Invest 121: 4056–4060.
25. Singh AB, Sharma A, Smith JF, Krishnan M, Chen X, et al. Claudin-1 up-regulates the repressor ZEB-1 to inhibit E-cadherin expression in colon cancer cells. Gastroenterology 141: 2140–2153.
26. Soler E, Andreu-Soler C, de Boer E, Bryne JC, Thomiwa S, et al. The genome-wide dynamics of the binding of Ldb1 complexes during erythroid differentiation. Genes Dev 24: 277–289.
27. Daniel JM, Spring CM, Crawford HC, Reynolds AB, Baig A (2002) The p120(catenin)-binding partner Kaiso is a bi-modal DNA-binding protein that recognizes both a sequence-specific consensus and methylated CpG dinucleotides. Nucleic Acids Res 30: 2911–2917.
28. Adachi Y, Yamamoto H, Ihoh F, Hinoda Y, Okada Y, et al. (1999) Contribution of the derived metastasis gene expression profile predicts recurrence and death in patients with colon cancer. Gastroenterology 138: 958–968.
30. Bloomston M, Zervos EE, Rosemurgy AS, 2nd (2002) Matrix metalloproteinases and their role in pancreatic cancer: a review of preclinical studies and clinical trials. Ann Surg Oncol 9: 608–674.

31. Liu D, Nakano J, Ishikawa S, Yokomise H, Ueno M, et al. (2007) Overexpression of matrix metalloproteinase-7 (MMP-7) correlates with tumor proliferation, and a poor prognosis in non-small cell lung cancer. Lung Cancer 58: 384–391.

32. Westermarck J, Kahanari VM (1999) Regulation of matrix metalloproteinase expression in tumor invasion. FASEB J 13: 781–792.

33. Crawford HC, Fingleton B, Gustavson MD, Kurpios N, Wagenaar RA, et al. (2001) The PEA3 subfamily of Ets transcription factors synergizes with beta-catenin-LEF-1 to activate matrixin transcription in intestinal tumors. Mol Cell Biol 21: 1379–1383.

34. Jiang G, Wang Y, Dai S, Liu Y, Stoecker M, et al. P120-catenin isoforms 1 and 3 regulate proliferation and cell cycle of lung cancer cells via beta-catenin and Kaiso respectively. PLoS One 7: e30303.

35. van Roy FM, McCrea PD (2005) A role for Kaiso-p120ctn complexes in cancer? Nat Rev Cancer 5: 956–964.

36. Yoon HG, Chan DW, Reynolds AB, Qin J, Wong J (2003) N-CoR mediates DNA methylation-dependent repression through a methyl CpG binding protein Kaiso. Mol Cell 12: 723–734.

37. Brabletz T, Jung A, Dac S, Hlubek F, Kirchner T (1999) beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. Am J Pathol 155: 1033–1038.

38. Kim EJ, Lee SY, Woo MK, Choi SI, Kim TR, et al. Fibulin-3 promoter methylation alters the invasive behavior of non-small cell lung cancer cell lines via MMP-7 and MMP-2 regulation. Int J Oncol 40: 402–408.

39. Chang MC, Chen CA, Chen PJ, Chiang VC, Chen YL, et al. Mesothelin enhances invasion of ovarian cancer by inducing MMP-7 through MAPK/ERK and JNK pathways. Biochem J 442: 293–302.

40. Stetler-Stevenson WG (1996) Dynamics of matrix turnover during pathologic remodeling of the extracellular matrix. Ann J Pathol 148: 1345–1350.

41. Coussens LM, Fingleton B, Marrasian LM (2002) Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 295: 2387–2392.

42. Hai T, Hartman MG (2001) The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor proteins and homeostasis. Gene 273: 1–11.

43. van Dam H, Castellazzi M (2000) Distinct roles of Jun: Fos and Jun: ATF dimers in oncogenesis. Oncogene 20: 2453–2464.

44. Kaszubska W, Hooft van Huijsduijnen R, Ghersa P, DeRaemy-Schenk AM, Chen BP, et al. (1993) Cyclic AMP-independent ATF family members interact with NF-kappa B and function in the activation of the E-selectin promoter in response to cytokines. Mol Cell Biol 13: 7180–7190.

45. Hui L, Bakiri L, Mairhofer A, Schweifer N, Haslinger C, et al. (2007) p38alpha suppresses normal and cancer cell proliferation by antagonizing the JNK-c-Jun pathway. Nat Genet 39: 741–749.

46. Ventura JJ, Tenbaum S, Perdiguero E, Huth M, Guerra C, et al. (2007) p38alpha MAP kinase is essential in lung stem and progenitor cell proliferation and differentiation. Nat Genet 39: 756–758.

47. Kajino T, Omori E, Ishii S, Matsumoto K, Ninomiya-Tsuji J (2007) TAK1 MAPK kinase kinase mediates transforming growth factor-beta signaling by targeting SnoN oncprotein for degradation. J Biol Chem 282: 9473–9481.