Inhibition of Wilms Tumor 1 Transactivation by Bone Marrow Zinc Finger 2, a Novel Transcriptional Repressor*

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The Wilms tumor suppressor gene, \textit{wt1}, encodes a zinc finger transcription factor that has been implicated in the regulation of a number of genes. Protein-protein interactions are known to modulate the transcription regulatory functions of Wilms tumor (WT1) and have also implicated WT1 in splicing. In this report, we identify a novel WT1-interacting protein, bone marrow zinc finger 2 (BMZF2), by affinity chromatography utilizing immobilized WT1 protein. BMZF2 is a potential transcription factor with 18 zinc fingers. The BMZF2 mRNA is mainly expressed in fetal tissues, and the protein is predominantly nuclear. Co-immunoprecipitation experiments are consistent with an in vivo association between WT1 and BMZF2. Glutathione S-transferase pull-down assays and far Western blots revealed that zinc fingers VI–X (amino acids 231–370) are required for interaction with the zinc finger region of WT1. Functionally, BMZF2 inhibits transcriptional activation by WT1. Moreover, a chimeric protein generated by fusion of BMZF2 to the GAL4 DNA-binding domain significantly decreases promoter activity of a reporter containing GAL4 DNA-binding sites, suggesting the presence of an active repressor domain within BMZF2. Our results suggest that BMZF2 interferes with the transactivation potential of WT1.

Wilms tumor (WT)\textsuperscript{1} is a pediatric kidney cancer, occurring with a frequency of 1 in 10,000 children, usually before the age of 5 years (1). It is thought to arise when multipotential cells of the metanephric blastema fail to differentiate and remain locked in a state of continual proliferation, and it has long been considered an excellent model for studying the relationship between cancer and development. A tumor suppressor gene, \textit{wt1}, implicated in predisposition to WT, has been extensively characterized and is mutated in 10–15\% of sporadic WTs (2). Germ line \textit{wt1} lesions in humans are associated with predisposition to WTs and aberrant differentiation of the urogenital system (3).

The \textit{wt1} gene encodes a transcription factor with a proline-glutamine-rich amino terminus and four carboxyl-terminal zinc fingers of the Krüppel C2-H2 class. The mRNA contains two alternative sites of translation initiation (4, 5), two alternatively spliced exons (6, 7), and undergoes RNA editing (8), thus potentially encoding 24 different protein isoforms with predicted molecular masses of 36–65 kDa. The function of the alternative translation initiation events, the RNA editing modification, and the first alternative splicing event (exon V) have not been well defined, although exon V can repress transcription when fused to a heterologous DNA-binding domain (9). Alternative splicing of exon IX inserts or removes three amino acids (\textit{\pm}KTS) (referred to as WT1(+)KTS or WT1(−)KTS) between zinc fingers III and IV and changes the DNA binding specificity of WT1 (10). The WT1(−)KTS isoforms can bind to two DNA motifs as follows: (i) a GC-rich motif, 5′ GXXGXXGGXG3′, related to the EGR-1-binding site (10); and (ii) a 5′CC3′, containing sequence (11). Recently NMR relaxation studies (12) have indicated that the KTS insertion increases the flexibility of the linker between fingers III and IV and abrogates binding of the fourth zinc finger to its cognate site in the DNA major groove. A number of genes involved in growth regulation and cellular differentiation contain WT1-binding sites within their promoters, and their expression can be modulated by WT1 in transfection assays (reviewed in Refs. 13–15).

The \textit{wt1} gene product has been shown to mediate both transcriptional repression and activation (reviewed in Refs. 13–15). Whether WT1 behaves as an activator or repressor appears to depend on promoter architecture surrounding the WT1-binding sites as well as on the presence of auxiliary transacting factors. Accordingly, it is well accepted that WT1 activity can be controlled by protein-protein interactions. A number of proteins are known to associate with WT1 and these include p53, p73, p63, SF-1, Par-4, Ciao 1, UBC9, Hsp70, U2AF65, CBP/p300, WTAP, and WT1 itself (reviewed in Refs. 13–15). The interaction of some of these proteins with WT1 is associated with modification of WT1 transcriptional properties as well as effects on the properties of the interacting partner (see “Discussion”). An additional role for WT1 in splicing is also postulated based on the subnuclear localization of WT1(+)KTS isoforms and the interaction of these isoforms with splicing factors (16–18).

Using affinity chromatography of nuclear extracts passed over immobilized WT1 protein, followed by mass spectrometric analysis of a specifically retained protein, we identified a novel WT1-interacting protein, named bone marrow zinc finger

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Novel WT1-interacting Zinc Finger Protein

EXPERIMENTAL PROCEDURES

Materials and General Methods—Restriction endonucleases, calf intestinal alkaline phosphatase, the Klenow fragment of DNA polymerase I, T4 DNA ligase, and T4 DNA polymerase were purchased from New England Biolabs. The luciferase assay kit was purchased from Promega. [32P]ATP (6000 Ci/mmol), [35S]methionine (>1000 Ci/mmol), 4-(dichloroacetyl)-1-L-tryptophan (54.0 Ci/mmol), [3H]CTP (21.8 Ci/mmol), and [32P]dCTP (3000 Ci/mmol) were from PerkinElmer Life Sciences.

Preparation of plasmid DNA, restriction enzyme digestion, agarose gel electrophoresis of DNA, DNA ligation, and bacterial transformation were carried out using standard methods (20). Clones of DNA PCR amplification products were always sequenced by the chain termination method using double-stranded DNA templates to ensure the absence of mutations.

Cell Culture, Transfections, and CAT and Luciferase Assays—293 and COS-7 cell lines were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal calf serum (In-vitrogen), penicillin, and streptomycin. For transient transfections, the cells were plated at a density of 2–5 × 10⁶ cells per 100-mm diameter dish 24 h prior to transfection. The cells were transfected by the calcium phosphate precipitation method (20). Individual DNA precipitates were adjusted to contain equal amounts of total DNA by the addition of the empty expression vector, pcDNA3. All transfections and subsequent CAT and luciferase assays were performed at least in duplicate. Cells were washed and refed 16 h post-transfection and harvested ~48 h later. Cells were scraped from the dishes following a phosphate-buffered saline (PBS) wash, centrifuged, and resuspended in 150 μl of 250 mM Tris (pH 8.0). They were then subjected to three rounds of freeze-thaw; an aliquot was taken for measurement of β-galactosidase activity, and the remainder of the extract was heated to 65 °C for 10 min and then assayed for CAT activity (21). Following thin layer chromatography, regions containing acetylated [14C]chloramphenicol, as well as unacetylated [14C]chloramphenicol, were quantitated by direct analysis on a PhosphorImager (Fujix BAS 2000). Luciferase activity was determined using the Promega luciferase assay kit. All CAT and luciferase activity values were normalized to β-galactosidase values, which served as internal controls in the transfections.

Plasmid Construction—The human BMZF2 cDNA was cloned by reverse transcriptase-PCR (RT-PCR) amplification from HeLa cells. The amplification primers used for this purpose were (i) 5'-th01 (5'-GATCTCTCAAGAGTGGTTCGA-3') XhoI site underlined) and 3'-th02 (5'-CATGACCGCCATTAAGGGAGACATCGCAA-3'); HindIII site underlined). The 5.8-kbp PCR product was digested with XhoI and HindIII and cloned into the same sites of pKSII+ to generate pKSII/BMZF2 (1–622). Deletion constructs of BMZF2 were prepared as follows. (ii) For pKSII/BMZF2 (1–80), the corresponding fragment was generated by PCR using primers 5'-th05 (5'-GATCTCTCAAGAGTGGTTCGA-3'; EcoRI site underlined) and 3'-th04 (5'-CCCAGGATCTCAATACAAAGGAGACATCGCAA-3') and ligated into the same sites of pKSII+. The PCR product was digested with EcoRI and BamHI and introduced into the same sites of pKSII+ to generate pKSII/BMZF2 (1–622). Deletion constructs of BMZF2 were prepared as follows. (iii) For pKSII/BMZF2 (1–80), the corresponding fragment was generated by PCR using primers 5'-th05 (5'-GATCTCTCAAGAGTGGTTCGA-3'; EcoRI site underlined) and 3'-th04 (5'-CCCAGGATCTCAATACAAAGGAGACATCGCAA-3'; BamHI site underlined) and ligated into the same sites of pKSII+. The PCR product was digested with EcoRI and BamHI and introduced into the same sites of pKSII+. (iv) For pKSII/BMZF2 (81–230), the corresponding fragment of the BMZF2 was generated by PCR using primers 5'-th06 and 3'-th08 (5'-GTTACTCGAGTTCCTTGCGCTGAAC-3'); XhoI site underlined). The fragment was digested with HindIII and XhoI and introduced into the same sites of pKSII+. (v) For pKSII/BMZF2 (1–230), the corresponding fragment was generated by PCR using primers 5'-th09 (5'-GTTACTCGAGTTCCTTGCGCTGAAC-3'); HindIII site underlined) and 3'-th07. The fragment was digested with HindIII and PstI and introduced into the same sites of pKSII+. (vi) For pKSII/BMZF2 (371–622), the corresponding fragment of the BMZF2 was generated by PCR using primers 5'-th10 (5'-GTTAAAGCTTACCATGATATAAGTG-3') and 3'-th11 (5'-GTTACTCGAGTTCCTTGCGCTGAAC-3'); PstI site underlined). The fragment was digested with HindIII and PstI and introduced into the same sites of pKSII+.

To generate amino-terminally hemagglutinin (HA)-tagged BMZF2 (CMV/BMZF2 (1–622)), the entire coding region of BMZF2 was excised from pKSII/BMZF2 (1–622) utilizing XhoI/XhoI, and this was inserted in-frame into the XhoI/XhoI site of plasmids HA-Eco12mer (a derivative of pcDNA3 containing three copies of the HA peptide epitope (HLA-YPFYDVPDYAG-COOH)) (kindly provided by H. Imataka and N. Sonenberg, McGill University). Plasmid CMV/BMZF2 (1–622) was constructed by PCR utilizing primers 5'-th12 (5'-GTTAAAGCTTACCATGATATAAGTG-3') and 3'-th12 (5'-GTTACTCGAGTTCCTTGCGCTGAAC-3'); HindIII site underlined). The PCR product was digested with HindIII and XhoI and cloned into the same sites of plasmids. Plasmid CMV/BMZF2 (1–623–370) was constructed by PCR-mediated mutagenesis with primers 5'-th14 (5'-GTTAAAGCTTACCATGATATAAGTG-3') and 3'-th14 (5'-GTTACTCGAGTTCCTTGCGCTGAAC-3'); XhoI site underlined) spanning an internal XhoI site and 5'-th15 (5'-GTTAAAGCTTACCATGATATAAGTG-3') and 3'-th15 (5'-GTTACTCGAGTTCCTTGCGCTGAAC-3'); XhoI site underlined). The PCR product was digested with HindIII and XhoI and cloned into the same sites of CMV/ BMZF2 (1–623–230). To generate CMV/GAL4/BMZF2, the BMZF2 coding region (amino acids 1–622) was removed from pKSII/BMZF2 (1–622) using HindIII and XhoI, replaced with the Klenow fragment of the BMZF2 coding region, and the XhoI site of pKSII/BMZF2 (1–622) was replaced with the yeast two-hybrid vector pGBK7T, producing pGBK7T/BMZF2 (1–622) and placing the Gal4 DNA domain upstream and in-frame with the entire BMZF2 coding region. The Gal4-BMZF2 fusion was excised from pGBK7T-BMZF2 utilizing HindIII and BamHI and inserted into the same sites of pcDNA3.

The construction and use of GST-WT1 bacterial expression vectors, eukaryotic expression vectors for WT1, the human vitamin D promoter reporter construct (–960 phvDVR/Luc), the Dax-1 reporter construct (pmDAX-L/CAT), the expression vector encoding the HA-tagged murine single-minded gene product (HA tagged SIM-2) (CMV/SIM-2), and the thymidine kinase (TK)-based reporters, pTECAT and pTECAT/TMPA, previously described, were utilized in the yeast two-hybrid assay. The yeast two-hybrid vector pGBK7T was purchased from Stratagene. The yeast lysate was centrifuged at 4 °C for 15 min at 1500 × g, and the supernatant was removed. The pellet was resuspended in 5.5 ml of 50 mM NaCl Affinity Chromatography (AC) Buffer (20 mM Hepes (pH 7.5), 10 mM MgCl₂, 0.5 mM DTT) supplemented with 1 mM benzamidine HCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, and 1 mM antipain. The cells were lysed using a type B Dounce, and lysis was verified by scraping the protoplasts with trypsin blue. The lysate was centrifuged at 4 °C for 30 min at 20,000 × g, and the supernatant was collected.

Preparation of HeLa Nuclear Extracts—Fifty (150-mm) plates of HeLa S3 cells were grown for the preparation of nuclear extracts. Cells were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 1-glutamine (4 mM) (Invitrogen) and grown at 37 °C in a humidified 5% CO₂ incubator. The cells were harvested by scraping in cold PBS and collected by centrifugation. After washing in PBS, the cell pellet was resuspended in 5 ml of Buffer A (10 mM Hepes (pH 7.9), 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM DTT) supplemented with 1 mM benzamidine HCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, and 1 mM antipain. The cells were lysed using a type B Dounce, and lysis was verified by scraping the protoplasts with trypsin blue. The lysate was centrifuged at 4 °C for 30 min at 20,000 × g, and the supernatant was collected.

Isolation of BMZF2 by Affinity Chromatography—GST and GST-21ZF1 WT1 zinc fingers I–IV fused in-frame to GST recombinant protein were coupled to Affi-Gel 10 at a protein/resin concentration of 0.5, and 2 mg/ml. Coupling reactions were performed in a final volume of 200 μl of AC buffer by incubating at 4 °C overnight and rotating the slurry of beads. The affinity matrix was washed in AC buffer containing 75 mM NaCl and 80 mM ethanolamine at room temperature for 1 h, followed by an additional 1 h wash in 75 mM NaCl/AC buffer containing 1 mg/ml purified bovine serum albumin. The matrix was then washed with AC buffer containing 1% NaCl at room temperature.
for 10 min and then equilibrated and stored in AC buffer containing 75 mM NaCl. Coupling efficiencies were ~80%. Ten column volumes (1 ml) of HeLa nuclear extract were applied to a small column containing 100 μl of affinity matrix. The resin was washed three times with AC buffer containing 75 mM NaCl. Elutions were performed sequentially with 2 times 2 column volumes (2 x 500 μl) of each of the following: (i) 75 mM NaCl-AC buffer with 1% Triton X-100; (ii) 300 mM NaCl-AC buffer; (iii) 1 M NaCl-AC buffer, and (iv) 1% SDS-AC buffer.

One-quarter of each fraction was analyzed on 12.5% SDS-polyacrylamide gels. Gels were prepared for silver staining by fixing overnight in 50% methanol, 10% acetic acid followed by a 10-min rinse in 20% ethanol and a 10-min rinse in water. Gels were then reduced with sodium thiosulfate (0.2 g/liter) for 1 min, rinsed twice with water for 20 s, and incubated in silver nitrate (in silver nitrate and formaldehyde) for 50 s, washed once with developing solution (sodium carbonate (30 g/liter), formaldehyde (1.4 ml of 37% solution/liter), sodium thiosulfate (10 mg/liter)) for 30 s and incubated in the developing solution until the desired intensity was reached. The reaction was stopped by exchanging the developing solution with 1% acetic acid for a minimum of 20 min. Speciﬁcally eluted bands were excised from the gel. The tryptic digestions of the protein samples were performed by Borealis Biosciences Inc., and the molecular mass of the tryptic fragments was determined with a Perspective Biosystems Voyager Elite MALDI-TOF (Toronto, Canada). The protein was identiﬁed by matching the observed proteolytic masses obtained in the MALDI-TOF spectra with the hypothetical tryptic masses derived from the NCBI non-redundant translated GenBank™ data base.

Native Co-Immunoprecipitations of BMZF2 and WT1—To express BMZF2 recombinant protein, we subcloned the amino-terminal non-zinc ﬁnger domain (amino acids 1–80) of BMZF from pKSII/BMZF2-(1–80) by digesting with BamHI and EcoRI and placing the coding fragment into the same sites of pGEX-6P-1 to produce pGEX-BMZF2. GST-BMZF2 was produced and puriﬁed according to the manufacturer’s recommendations (Promega). The GST domain was remove by digesting with PreScission Protease (Promega) overnight at 4 °C, followed by passing of the material through a second glutathione afﬁnity matrix. Rabbis were immunized with BMZF2-(1–80) (4). Affinity-purified antibodies were obtained by using immunosafinity columns containing BMZF2-(1–80) immobilized to Affi-Gel 10 resin (Amer sham Biosciences).

For the analysis of the interaction between endogenous WT1 and BMZF2, K562 cells were lysed in lysis buffer (20 mM Tris-HCl (pH 7.4), 10 mM KCl, 10 mM MgCl₂, 2 mM EDTA, 10% glycerol, 1% Triton X-100, 2.5 mM β-glycerol phosphate, 1 mM NaF, 1 mM DTT, 1 μg/ml of aprotinin, 1 μg/ml of leupeptin, 1 μg/ml of Pefabloc, and 1 μg/ml of pepstatin A) for 10 min on ice. Lysates were sonicated twice for 15 s and incubated with 420 μg NaCl in lysis buffer for 1 h on ice. Extracts were incubated with the indicated antisera or antibodies, and immune complexes were collected with protein A-Sepharose beads at 4 °C for 1 h. The beads were washed three times with lysis buffer, and proteins were separated by SDS loading buffer. Immunoprecipitates were detected by SDS-PAGE and detected by Western blotting using against anti-WT1 antibody (F-6, Santa Cruz Biotechnology) or anti-BMZF2 antibody.

Northern Blotting—Northern blotting was carried out on commercially available blots according to the manufacturer’s recommendation (Promega). Blots were probed with 32P-labeled human BMZF2 cDNA probe (240-bp fragment obtained by BamH1/EcoR1 digest of pKSII/BMZF2-(1–80) (~9 x 10⁶ cpm/μg) and a human β-actin probe (~1.0-kbp fragment obtained by EcoRI/XhoI digest of pKSII-β-actin (~9 x 10⁶ cpm/μg)). Both probes had been prepared by random priming (20). Probing was performed at a probe concentration of 10⁶ cpm/ml at 65 °C in 1 x SSPE buffer (50 mM sodium formate, 5 x SSPE (1 x SSPE is 0.15 M NaCl, 0.01 μM NaH₂PO₄, 1 mM EDTA, pH 7.4), 2 x Denhardt’s reagent, 0.1% SDS, 100 μg of denatured, fragmented salmon sperm DNA/ml). The blot was then washed once in 2 x SSC, 0.05% SDS at room temperature and twice in 0.1 x SSC, 0.1% SDS at 50 °C, and subjected to autoradiography.

Subcellular Localization of BMZF2 and WT1—293 cells were co-transfected with 10 μg of CMV/WT1 (−/−) together with 10 μg of pcdNA3, CMV/BMZ2F-(1–622), CMV/Par-4, or CMV/SIM DNA. After 48 h, cells were lysed in lysis buffer (25 mM Hepes (pH 7.4), 157 mM NaCl, 1% Triton X-100, 10 mM β-glycerol, 2.5 mM EDTA, 2.5 mM EGTA, 5 mM β-glycerol phosphate, 1 mM NaVO₃, 1 μg/ml of aprotinin, 1 μg/ml of leupeptin, 1 μg/ml of Pefabloc, 1 μg/ml of pepstatin A, 1 μg/ml of 1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone, 1 μg/ml of 1-chloro-3-(tosylamido)-7-aminotriazine-2,6-heptane, 5 mM NaF, and 5 mM sodium pyrophosphate) for 30 min on ice. Extracts were incubated with anti-WT1 antibodies (C-19, Santa Cruz Biotechnology) overnight, and immune complexes were collected with protein G-Sepharose beads at 4 °C for 1 h. The beads were washed 6 times with lysis buffer, and the proteins were eluted with 1 x SDS loading buffer. Proteins were separated by electrophoresis through a 10% SDS-polyacrylamide gel and transferred to an Immobilon-P membrane (Millipore). After blocking with 5% skim milk in PBS, the membrane was incubated for 1 h with anti-HA antibody (1:1000) (HA.11, Babco). The membrane was washed in PBS, and antibody binding was visualized with peroxidase-conjugated goat anti-mouse secondary antibody (1:5000) (Amersham Biosciences) utilizing ECL reagents (Amersham Biosciences).

Immunoprecipitation Assays—293 cells were co-transfected with 10 μg of CMV/WT1 (−/−) and 10 μg of pcdNA3, CMV/BMZ2F-(1–622), CMV/Par-4, or CMV/SIM DNA. After 48 h, cells were lysed in lysis buffer (25 mM Hepes (pH 7.4), 157 mM NaCl, 1% Triton X-100, 10 mM β-glycerol, 2.5 mM EDTA, 2.5 mM EGTA, 5 mM β-glycerol phosphate, 1 mM NaVO₃, 1 μg/ml of aprotinin, 1 μg/ml of leupeptin, 1 μg/ml of Pefabloc, 1 μg/ml of pepstatin A, 1 μg/ml of 1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone, 1 μg/ml of 1-chloro-3-(tosylamido)-7-aminotriazine-2,6-heptane, 5 mM NaF, and 5 mM sodium pyrophosphate) for 30 min on ice. Extracts were incubated with anti-WT1 antibodies (C-19, Santa Cruz Biotechnology) overnight, and immune complexes were collected with protein G-Sepharose beads at 4 °C for 1 h. The beads were washed 6 times with lysis buffer, and the proteins were eluted with 1 x SDS loading buffer. Proteins were separated by electrophoresis through a 10% SDS-polyacrylamide gel and transferred to an Immobilon-P membrane (Millipore). After blocking with 5% skim milk in PBS, the membrane was incubated for 1 h with anti-HA antibody (1:1000) (HA.11, Babco). The membrane was washed in PBS, and antibody binding was visualized with peroxidase-conjugated goat anti-mouse secondary antibody (1:5000) (Amersham Biosciences) utilizing ECL reagents (Amersham Biosciences).

RESULTS

Isolation of BMZF2 as a Novel WT1-interacting Protein—Genetic screens and chemical cross-linkers have identiﬁed a small number of proteins that interact with the WT1 zinc ﬁnger domain (see “Discussion”). We looked to employ a biochemical approach to validate the interaction of previously described WT1-interacting proteins, as well as identify potentially new protein(s) that could interact with the WT1 zinc ﬁnger domain. To this end, HeLa nuclear extracts were incubated with Affi-Gel 10 resin that had been coupled to GST or GST-WT1ZF fusion proteins. Elutions obtained with 300 mM...
NaCl, 1 M NaCl, and 1% SDS were analyzed by SDS-PAGE and silver staining. Two proteins, an 40-kDa (denoted by an asterisk) and 30-kDa (denoted by an arrow) species, were visible in the 1 M NaCl elution (Fig. 1A). The 40-kDa species is present in elutions from both the GST and GST-WT1ZF affinity matrices (Fig. 1A, lanes 2, 3, and 5–7) and was not observed if HeLa nuclear extracts were incubated with 0, 0.5, and 2.0 mg/ml Affi-Gel 10 resin coupled to GST or GST-WT1ZF. Following elution with 1 M NaCl, 40% (25%) of each eluent was analyzed on a 12.5% SDS-polyacrylamide gel and visualized by silver staining. The position of migration of an 40- and an 30-kDa protein species is indicated by an asterisk and an arrowhead, respectively. The protein to resin cross-linking ratio, as well as whether or not nuclear extract had been applied to the affinity matrix, is indicated above the panel. The positions of migration of molecular mass markers (New England Biolabs) is indicated to the left. B, nucleotide and amino acid sequence of BMZF2 cDNA. The nucleotide and amino acid sequences of BMZF2 are shown with numbering of nucleotides and amino acids displayed to the right. The matched BMZF2 peptides identified by MALDI-TOF are in rectangles. The zinc fingers are shown in boldface type. The amino acid sequence differences between our sequence and that in GenBank™, accession numbers NM 005774 and AF067164, are highlighted by an underline.
nuclear extract were omitted from the GST or GST-WT1ZF columns (Fig. 1A, lanes 1 and 4). These results suggest that the 40-kDa protein species is not specifically retained by the WT1ZF domain, and characterization of this species was not further pursued. The ~30-kDa protein species was observed only in elutions from the GST-WT1ZF matrix (compare lanes 6 and 7 to lane 3), was not observed in elutions from columns that had no GST-WT1ZF coupled to them but had been exposed to nuclear extract (lanes 2 and 5), and was not observed in elutions where the GST-WT1ZF column had not been exposed to nuclear extract (lane 4). As well, this protein species was not observed in the 300 mM NaCl elutions from the GST-WT1ZF column (data not shown). These results indicate that the ~30-kDa protein species was specifically retained by and eluted from the GST-WT1ZF affinity matrix.

Mass spectrometry of 15 peptides obtained from the excised protein from the SDS gel identified two proteins, 9 peptides matched to BMZF2 and will be the focus of the current study. The experimentally determined masses of the 9 peptides matched bone marrow zinc finger 2 (denoted by gray boxes in Fig. 1B) and are in good agreement with the predicted sizes (shown in italics): 1475.7 Da (1474.8, 1642.7 Da (1641.8, 1166.5 Da (1167.6, 1463.7 Da (1462.8, 1638.8 Da (1637.8, 2201 Da (2201.7, 1707.8 Da (1706.8, 1475.8 Da (1476.8, and 1257.6 Da (1256.7). In order to pursue functional studies with BMZF2, we used RT-PCR to obtain a full coding version of the gene from HeLa cell mRNA (Fig. 1B). BMZF contains a Krüppel-related amino-terminal domain, named the Krüppel-related novel box (KNRB), and 18 Krüppel-like zinc fingers (19). These features suggest that BMZF2 is a nucleic acid-binding protein with potential transcriptional activity.

Sequence analysis of our clones, as well as PCR products, revealed several discrepancies between our sequence and the BMZF2 sequence deposited in GenBankTM (GenBank™ accession numbers NM005774 and AF067164). The reported sequence indicates the presence of 2 adenosine residues at nucleotides 153 and 154, 2 adenine residues at nucleotides 192 and 193 (on our sequence nucleotide 191), and 2 thymidine residues at nucleotides 200 and 201 (on our sequence nucleotide 198), whereas we find a single adenine and thymidine residue at these corresponding positions. This has the net effect of altering the reading frame of a portion of the amino-terminal domain of BMZF2 (denoted by an underline in Fig. 1B) and reducing the size of the predicted BMZF2 protein product by 1 amino acid. These differences may reflect errors in the reported sequence of BMZF2 or alternative splicing events. Additionally, there is an adenine residue at position 946 (corresponds to a thymidine at our position 943), a cytosine at position 949 (corresponds to an adenine at our position 946), a thymidine at position 983 (corresponds to an adenine residue at our position 980), and a cytosine at position 1309 (corresponds to a thymidine at position 1306). The amino acids altered by these differences are underlined in the sequence presented in Fig. 1B and may reflect sequencing errors in the original sequence or polymorphisms. We find these same sequence differences in RT-PCR products obtained from RNA isolated from non-transformed HeLa cells (data not shown).

In Vivo and In Vitro Interaction between BMZF2 and WT1—

There was a clear discrepancy in masses between the ~30-kDa protein species identified by affinity chromatography and the predicted mass for BMZF2 (72 kDa). To resolve this, we performed co-immunoprecipitation experiments from extracts prepared from human K562 erythroleukemia cells that express both WT1 (26) and BMZF2 (19) (Fig. 2). Immunoprecipitation with either non-immune rabbit serum (Fig. 2A, lane 1) or a rabbit polyclonal anti-BMZF2 antibody (Fig. 2A, lane 2) was performed on extracts prepared from K562 cells. Following fractionation of the immunoprecipitates by SDS-PAGE, Western blotting analysis was performed using an anti-WT1 antibody (Fig. 2A). The presence of an immunoreactive protein species of ~50 kDa was detected only when anti-BMZF2 was used as the immunoprecipitating antibody (compare lane 2 to 1) and is consistent with WT1 isoforms generated from the first ATG initiation codon (4). When anti-BMZF2 antibodies were used to probe total cell extracts from K562 cells (Fig. 2B, lane 3), 6 immunoreactive protein species were visible, ranging in size from ~25 to 72 kDa. These protein species are either cross-reacting with our antibody preparation or represent
BMZF2 isoforms that contain a portion of the BMZF2 amino terminus (because our polyclonal was raised against the first 80 amino acids of the protein). Immunoprecipitations utilizing either an α-HA antibody (as negative control) (Fig. 2B, lane 1) or a monoclonal α-WT1 antibody (Fig. 2B, lane 2), followed by probing for the presence of BMZF2 revealed that four of these species were retained by WT1 (compare lane 2 to 1). We note that one of these species is ~30 kDa in molecular mass and likely corresponds to the species that we initially identified by affinity chromatography (Fig. 1A).

To confirm these results, co-immunoprecipitation experiments were conducted with extracts prepared from 293 cells transiently transfected with expression vectors driving synthesis of WT1 and HA-tagged BMZF2, Par-4, and SIM-2. After transfections, cells were lysed, immunoprecipitated with a polyclonal anti-WT1 antibody, and subjected to Western blot analysis utilizing an anti-HA antibody. In this experiment, Par-4, a protein known to interact with WT1 (27), acts as our positive control, whereas SIM-2, a member of the PAS (Per-Grn-Sim) family of transcription factors and not known to interact with WT1, acts as our negative control. As shown in Fig. 2C, Par-4 is pulled down from cells with WT1 (compare lane 4 to 1), whereas SIM2 is not (compare lane 3 to 1). BMZF2 was co-immunoprecipitated with WT1 (compare lane 5 to 1). Co-immunoprecipitation of BMZF2 was not due to cross-reactivity of BMZF2 with the anti-WT1 antibodies, because BMZF2 was not present in immunoprecipitation reactions performed with this antibody on extracts that lacked WT1 (lane 2). Taken together, these results indicate that WT1 and BMZF2 interact in vivo.

We performed in vitro pulldown assays to determine the protein region(s) required for BMZF2 and WT1 interaction. Luciferase, BMZF2, and p53 were produced in in vitro translation systems and tested for their ability to bind to GST-WT1Zf immobilized on glutathione resin. As expected, luciferase did not bind to immobilized GST or GST-WT1Zf (Fig. 3A, compare lanes 4 and 7 to lane 1). p53, a factor known to interact with WT1, was specifically retained by immobilized GST-WT1Zf but not by the GST affinity column (compare lane 9 to 6). Similarly, BMZF2 was retained by GST-WT1Zf but not by GST (compare lane 8 to 5). A set of BMZF2 deletion mutants was generated and used to map the region responsible for the interaction with WT1Zf (Fig. 3B).WT1Zf bound to BMZF2-(81–622) (lacking the first 80 amino-terminal amino acids) but not to BMZF2-(1–80), indicating that the amino-terminal 80 amino acids are not responsible for the binding to WT1Zf (Fig. 3B).

WT1Zf also bind to deletion mutants BMZF2-(81–370) and BMZF2-(231–370) but not to BMZF2-(371–622) or BMZF2-(81–230), indicating that amino acids 231–370 (zf fingers 6–10) contain the WT1-binding site (Fig. 3B).

Far Western blotting was also used to confirm the interaction between WT1 and in vitro translated 35S-labeled BMZF2. GST-WT1(1–446), GST-WT1(1–242), and GST-WT1-(297–446) (Fig. 3C) were purified, fractionated by SDS-PAGE, transferred onto a PVDF membrane, and incubated with 35S-labeled BMZF2. Although GST-WT1(1–446) and GST-WT1-(297–446) bound to BMZF2, GST-WT1(1–242) was unable to bind to BMZF2 (Fig. 3D, upper panel). Blotting with anti-WT1 antibodies demonstrated similar amounts of GST-WT1 protein loaded in all lanes (Fig. 3D, lower panel). These results indicate that the WT1 zinc fingers are sufficient for interaction with BMZF2.

Expression of BMZF2 mRNA in Human Tissues and Subcellular Localization of BMZF2—To determine the expression pattern of BMZF2 mRNA, we analyzed adult and fetal human mRNAs from a variety of tissues by Northern blotting. To avoid cross-hybridization to other zinc finger transscripts, the blots were probed with the amino-terminal domain of BMZF2 (non-zinc finger domain). We could not detect any expression of BMZF2 in adult tissues (data not shown). We could only detect BMZF2 expression in fetal tissues, where we observed three major transcripts. Two of these transcripts, of ~5.0 and ~4.0 kb, were detected in fetal brain, lung, liver, and kidney (Fig. 4A). A shorter transcript of ~3.4 kb was detected in fetal lung tissue (Fig. 4A). We have not determined the structure of the three different transcripts, but these may arise from alternative transcription initiation, splicing, or differential use of polyadenylation sites. This raises the possibility that several protein isoforms may exist for BMZF2. As a control for the amount of RNA present in each lane, we reprobed the Northern blot with a human β-actin probe (Fig. 4A).

The subcellular localization of BMZF2 was also examined. CMV/BMZF2-(1–622) and CMV/WT1(−/−) were transfected into 293 cells. Cells were lysed, and cytosolic and nuclear protein fractions were prepared and subjected to SDS-PAGE and Western blot analysis. As shown in Fig. 4B, BMZF2 and WT1 were present in the nuclear fraction (Fig. 4B, upper and middle panel). As a further control for the quality of the fractionation procedure, a cytoplasmic protein, Grb2 (28), was detected only in the cytoplasmic fraction (Fig. 4B, lower panel).

Immunofluorescence analysis of transfected H1299 cells with CMV/BMZF2-(1–622) revealed the presence of BMZF2 in the nucleus (data not shown). Taken together, these results identify BMZF2 as a nuclear protein.

Inhibition of WT1-mediated Activation by BMZF2—To investigate the functional consequences of the WT1-BMZF2 interaction, we examined whether introduction of BMZF2 would affect transcriptional activation by WT1. WT1(−/−)KTS isoforms have been shown previously (25) to activate a reporter construct containing a WT1-binding site within the human vitamin D receptor (VDR) promoter. A series of reporter and expression constructs were utilized to analyze the effect of BMZF2 on the functional properties of WT1 (Fig. 5A). When ~960phVDR/Luc was transfected with the empty expression vector pcDNA3, very little luciferase activity was observed (Fig. 5B, lane 2). Transfection with CMV/WT1(−/−)KTS resulted in a 5.2-fold activation of the VDR promoter (Fig. 5B, lane 3), similar to results reported previously (25). Transfection of CMV/BMZF2-(1–622) with ~960phVDR/Luc did not significantly affect the levels of luciferase produced from ~960phVDR/Luc (lane 4) indicating that under these conditions BMZF2 does not affect expression from the VDR promoter.

Transfection of increasing amounts of CMV/BMZF2(1–622) resulted in a dose-dependent decrease in WT1-mediated transcriptional activation (Fig. 5B, compare lanes 2 to 5). Western blotting of nuclear extracts from the transfected cells demonstrated that increasing amounts of CMV/BMZF2(1–622) were synthesized in response to increasing amounts of transfected plasmid (Fig. 5C). These results indicate that BMZF2 can inhibit WT1-mediated activation.

To ensure that the observed results were not specific to the human VDR promoter, we assessed the ability of BMZF2 to mediate repression of WT1 activation on a different reporter system, one that employed the murine Dax-1 promoter (24). Co-transfection of CMV/WT1(−/−) with pmDAX-1/CAT results in a 12-fold increase in CAT expression (Fig. 5D, compare lane 3 to 2). Co-transfection of CMV/BMZF2(1–622) and pmDAX-1/CAT did not significantly alter production of CAT from the Dax-1 promoter (compare lane 4 to 2). Transfection of increasing amounts of CMV/BMZF2(1–622) resulted in a dose-dependent decrease in WT1-mediated transcriptional activation (Fig. 5D, compare lanes 5–8). Our results indicate that BMZF2 is also capable of repressing...
Fig. 3. **Association of BMZF2 with WT1.** A, GST pulldown assays of BMZF2 with GST-WT1ZF. An aliquot of the input (10%) [35S]methionine-labeled protein (lanes 1–3) used in the pulldown assay, the pellet from the GST pulldown (lanes 4–6), or the pellet from the GST-WT1ZF pulldown (lanes 7–9) were fractionated on a 10% SDS-PAGE. The gel was treated with EN3HANCE and dried, and proteins were visualized by autoradiography. Recombinant proteins used were [35S]-labeled luciferase (lanes 1, 4, and 7), BMZF2 (lanes 2, 5, and 8), and p53 (lanes 3, 6, and 9). The positions of migration of BMZF2 and p53 are indicated by arrows to the right. The positions of molecular mass markers (New England Biolabs) are indicated to the left. Note that the p53 product shows two bands in the input (lane 3), the lower molecular weight species may be due to internal translation initiation. B, mapping of the WT1 interacting domain of BMZF2. The black box represents the BMZF2 amino-terminal domain (KRNB), and the zinc fingers are represented by individual open boxes. The amino acid position of domains of BMZF2 are shown above the schematic.
activated transcription mediated by WT1(−/−) from the Dax-1 promoter as well (Fig. 5D).

To demonstrate that BMZF2 binding to WT1 was required to obtain the observed effects on WT1-mediated transcriptional activation, we constructed a BMZF2 deletion mutant that lacks the WT1-binding domain (amino acids 231 to 370), called CMV/BMZF2-(Δ231–370). As with CMV/BMZF2-(1–622), transfection of CMV/BMZF2-(Δ231–370) with −960phVDR/Luc had little effect on VDR promoter activity (Fig. 5E, compare lane 4 to 5). Whereas transfection of CMV/BMZF2-(1–622) with CMV/WT1(−/−) inhibited WT1-mediated activation (lane 6), CMV/BMZF2-(Δ231–370) did not (lane 7). Taken together, these results suggest that BMZF2 specifically inhibits WT1-mediated transcriptional activation through its physical association with WT1.

**BMZF2 Is a Transcriptional Repressor**—To assess the transcriptional properties of BMZF2, BMZF2 was fused to the Gal4 DNA-binding domain, generating CMV/GAL4/BMZF2. Two reporter plasmids were used in these experiments, pTECAT/5XGAL4, which contains five GAL4-binding sites upstream of the TK minimal promoter, and pTECAT, which lacks GAL4-binding sites and serves as a negative control (Fig. 6A). An expression vector driving the synthesis of only the GAL4 DNA-binding domain, CMV/GAL4, had no effect on the levels of CAT produced from either pTECAT or pTECAT/5XGAL4 when transfected into COS-7 cells (Fig. 6B, lanes 2–5 and 11–14). On the representation of the constructs. [35S]Methionine-labeled BMZF2 and deletion mutants produced by in vitro translation were incubated with immobilized GST-WT1ZF. Following washing, the bound proteins were eluted with SDS loading buffer, and proteins were visualized by autoradiography. The + or − symbols to the right refer to the ability or inability, respectively, to bind to GST-WT1ZF. C, schematic representation of GST-WT1 truncation mutants. The first alternative splice site (exon V) consists of 17 amino acids (VAAGSSSSVKWTEGDSN). The amino acid position of the WT1 regions are shown below the schematic representation of the constructs. The open box represents the GTS domain; the black box represents the non-zinc finger domain of WT1, and the WT1 zinc fingers are denoted by individual boxes. D, BMZF2 directly interacts with WT1 zinc finger domain. Bacterially produced GST-WT1 proteins were fractionated on a 10% SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked in PBST containing 5% skim milk, followed by incubation with [35S]methionine-labeled BMZF2 protein for 2 h at 4 °C. After four washes in PBST, the bound BMZF2 was detected by fluorography (upper panel). Probing for GST-WT1 recombinant protein by Western blotting was used to qualify the GST-WT1 recombinant proteins (lower panel). The positions of molecular mass markers (New England Biolabs) are indicated to the left.
other hand, CMV/GAL4/BMZF2 repressed CAT production from pTECAT/5XGAL4 in a dose-responsive manner (lanes 6–9) but did not affect expression from pTECAT (lanes 15–18), indicating that repression by CMV/GAL4/BMZF2 is dependent on the presence of GALA4-binding sites within the reporter vector. Additionally, CMV/BMZF2(1–622), which lacks a GALA4 DNA-binding domain, did not inhibit CAT expression from pTECAT/5XGAL4 (Fig. 6C, compare lane 4 to 3 and 2), consistent with the need for BMZF2 to bind to the reporter construct to achieve repression of transcription. These results demonstrate that BMZF2 is capable of repressing transcription.

**DISCUSSION**

The identification of BMZF2 as a WT1 interacting partner was achieved by affinity chromatography of HeLa extracts on immobilized WT1ZF. Although the BMZF2 transcript should produce a protein of 72 kDa, we identified BMZF2 through mass spectrometry analysis of a 30-kDa protein species (Fig. 3B, lane 4, compare with lane 3). Furthermore, BMZF2 mRNA levels are not increased in HeLa cells upon WT1 downregulation (Fig. 3A, compare lane 4 to lane 3), indicating that BMZF2 is not upregulated in WT1-repressed cells.

To determine the function of BMZF2 in repressing WT1-mediated transcription, we performed transfections in COS-7 cells expressing WT1, WT1ZF, or BMZF2 alone or in combination (Fig. 4). WT1ZF repressed CAT activity when cotransfected with pRSV/β-galactosidase (Fig. 4B, lane 2 vs. lane 1), consistent with the need for WT1 to bind to the reporter construct to achieve repression of transcription. The repression by WT1ZF is specific, as BMZF2 alone did not affect CAT expression (Fig. 4B, lane 3 vs. lane 1). These results demonstrate that BMZF2 is capable of repressing transcription.

**FIG. 5. BMZF2 inhibits WT1-mediated transcriptional activation.** A, reporter and expression plasmids used in this study. The open box represents the human VDR promoter, the black box denotes the firefly luciferase coding region, and the gray box symbolizes the CAT coding region. The box with the radial shading represents the murine Dax-1 promoter. The box with the horizontal gradient represents the WT1 non-zinc finger domain with the 4 individual zinc fingers denoted by open boxes. The BMZF2 coding region is represented by a box with vertical shading. B, transfections in 293 cells were performed with increasing amounts of CMV/BMZF2(1–622) in the presence of 5 μg of CMV/WT1(−/−), 1 μg of −960phVDR/Luc reporter plasmid, and 1 μg of pRSV/β-galactosidase (lane 2), which was set at 1. The total transfected DNA concentration was kept constant by the addition of the empty expression vector, pcDNA3, to make up for differences in amounts between transfections. The error bars represent the S.E. of three separate experiments, with each sample transfected in duplicate in each experiment. C, analysis of BMZF2 in transfected cells. Extracts used for luciferase assays in B were resolved on a 10% SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with anti-HA antibody (HA.11; Babco). D, WT1-mediated activation of the murine Dax-1 reporter construct is inhibited by BMZF2. Transfections in COS-7 cells were performed with increasing amounts of CMV/BMZF2(1–622) in the presence of 10 μg of CMV/WT1(−/−) and 1 μg of pmDAX-1/CAT. The total transfected DNA concentration was kept constant by the addition of the empty expression vector, pcDNA3, to make up for differences in amounts between transfections. To normalize for transfection efficiency, the cells were co-transfected with 1 μg of pRSV/β-galactosidase (lane 3), which was set at 1. Ac-Cm, acetylated chloramphenicol; Cm, chloramphenicol; O, origin. E, a BMZF2 mutant lacking the WT1-interacting domain does not affect WT1-mediated transcriptional activation. The human VDR reporter construct, −960phVDR/Luc was transfected alone (lane 2) or with CMV/WT1(−/−) (lane 3), CMV/BMZF2(1–622) (lane 4), CMV/BMZF2(Δ231–370) (lane 5), CMV/BMZF2(Δ231–370) and CMV/WT1(−/−) and CMV/BMZF2(1–622) (lane 6), or CMV/WT1(−/−) and CMV/BMZF2(Δ231–370) (lane 7). The amounts of transfected plasmids are indicated below the lanes. The total transfected DNA concentration was kept constant by the addition of the empty expression vector, pcDNA3, to make up for differences in amounts between transfections. The error bars represent the S.D. of three independent experiments, with each sample transfected in duplicate. Luciferase activity of each transfection was set relative to the activity obtained by transfecting pcDNA3, −960phVDR/Luc, and pRSV/β-galactosidase (lane 2; which was set at 1).
FIG. 6. BMZF2 is a transcriptional repressor. A, schematic representation of the pTECAT and pTECAT/5XGAL4 reporter constructs used in this experiment. The white box denotes the thymidine kinase (TK) promoter, the box with the gray gradient represents the 5 GAL4-binding sites, and the CAT open reading frame is illustrated by a black box. The right-angled arrow indicates the transcription start site. Nucleotide positions demarcate the boundaries of the TK promoter, which are identical in the two reporter constructs. B, COS-7 cells were co-transfected with the indicated amounts of pTECAT/5XGAL4 or pTECAT and increasing amounts of CMV/GAL4 or CMV/GAL4/BMZF2 vector. The amounts of transfected plasmids are indicated below the lanes. The total transfected DNA concentration was kept constant by the addition of the empty expression vector, pcDNA3, to make up for differences in amounts between transfections. The error bars represent the S.E. of three separate experiments, with each sample transfected in duplicate. CAT activity of each transfection was set relative to the activity obtained by transfecting pcDNA3, pTECAT/5XGAL4, and pRSV/β-galactosidase (lane 1; which was set at 1). C, COS-7 cells were co-transfected with the indicated amounts of pTECAT/5XGAL4 and CMV/GAL4, CMV/GAL4/BMZF2, or CMV/BMZF2(1–622) expression vector. The amounts of transfected plasmids are indicated below the lanes. The total transfected DNA concentration was kept constant by the addition of the empty expression vector, pcDNA3, to make up for differences in amounts between transfections. To normalize for transfection efficiency, the cells were co-transfected with 1 μg of pRSV/β-gal. At 48 h after transfection, the cells were harvested and assayed for β-galactosidase and CAT activity. The average fold activation and S.E. for CAT determinations are indicated below the representative chromatogram and represent the value obtained from two independent experiments. CAT activity of each transfection was set relative to the activity obtained by transfecting pcDNA3, pTECAT/5XGAL4, and pRSV/β-galactosidase (lane 1; which was set at 1). Ac-Cm, acetylated chloramphenicol; Cm, chloramphenicol; O, origin.
BMZF2 is expressed in fetal brain, lung, liver, and kidney (Fig. 4). We failed to detect any expression by Northern blotting in adult tissues (Fig. 4). Although Han et al. (19) reported that BMZF2 is expressed to low levels in the heart, brain, lung, kidney, testis, bone marrow, liver, spleen, pancreas, stomach, placenta, and in six leukemic cell lines, they had to resort to RT-PCR to detect expression in these tissues. It is tempting to speculate that BMZF2 is a developmentally regulated transcriptional repressor that is not present in many adult tissues or is present to very low levels in these tissue. We observed three transcripts (3.4, 4, and 5 kb) by Northern blotting of RNA isolated from fetal tissue utilizing the unique amino-terminal domain of BMZF2 as a probe and under stringent hybridization and wash conditions. The 3.4-kb transcript was present in lung tissue and absent in brain, liver, and kidney. The other two transcripts were present in all four tissues. We have yet to explain the underlying structure features that are responsible for generating these three different transcripts.

Hematopoiesis is a complex physiological process that requires intricate regulation of gene expression during embryogenesis, fetal life, and adult life. BMZF2 was first identified from an acute promyelocytic leukemia cell line, NB4 and is expressed in a number of leukemia cell lines (19). WT1 is expressed in early bone marrow precursors and rapidly downregulated following differentiation of these cells and leukemia-derived cell lines, suggesting that it may also play a role in early hematopoiesis (38–41). WT1 is also highly expressed in many human acute leukemias, suggesting that mis-expression of WT1 may also be a contributor to hematopoietic malignancies (42, 43). Recently, WT1 has also been shown to induce growth arrest and differentiation in primary hematopoietic progenitors (44). Conversely, the loss of act1 gene function has also been implicated in the development of malignancies including acute leukemias (45). This correlates with the tumor-suppressive effects of WT1 expression in leukemia cell lines and suggests that WT1 acts as a differentiation-promoting gene during hematopoiesis and that the loss of functional WT1 expression may contribute to leukemogenesis. Understanding the molecular relationship between WT1 and BMZF2 may provide insight into the role of these two proteins in normal hematopoiesis, as well as understanding events that become deregulated during the development of hematopoietic malignancies.

**REFERENCES**

1. Matsunaga, E. (1981) *Hum. Genet.* 57, 231–246
2. Varanasi, R., Bardeesy, N., Ghahremani, M., Petruzzii, M. J., Nowak, N., Adam, M. A., Grundy, P., Shows, T. B., and Pelletier, J. (1994) *Proc. Acad. Sci. U. S. A.* 91, 3554–3558
3. Pelletier, J., Bruening, W., Kashtan, C. E., Mauer, S. M., Manivel, J. C., Sigel, J. E., Houghton, M. C., Junien, C., Habib, R., Fauser, L., Fine, R. N., Silverman, B. L., Haber, D. A., and Houssman, D. (1991) *Cell* 67, 437–447
4. Bruening, W., and Pelletier, J. (1996) *J. Biol. Chem.* 271, 8646–8654
5. Scharnhorst, V., Dekker, P., van der Eb, A. J., and Jochemsen, A. G. (1999) *J. Biol. Chem.* 274, 23456–23462
6. Haber, D. A., Buckler, A. J., Glaser, T., Call, K., Pelletier, J., Sohn, R., Douglass, E., and Houssman, D. E. (1990) *Cell* 61, 1257–1269
7. Haber, D. A., Sohn, R. L., Buckler, A. J., Pelletier, J., Call, K. M., and Houssman, D. E. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 9618–9622
8. Sharma, P. M., Bowman, M., Madden, S. L., Rauscher, F. J., III., and Sukumar, S. (1994) *Genes Dev.* 8, 720–731
9. Wang, Z. Y., Qiu, Q. Q., Huang, J., Gurrieri, M., and Deuel, T. F. (1995) *Oncogene* 10, 415–422
10. Rauscher, F. J., III, Morris, J. F., Tournay, O. E., Cook, D. M., and Curran, T. (1990) *Science* 250, 1259–1262
11. Wang, Z. Y., Qiu, Q. Q., Enger, K. T., and Deuel, T. F. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 8896–8900
12. Laity, J. H., Dyson, H. J., and Wright, P. E. (2000) *Proc. Natl. Acad. Sci. U. S. A.* 97, 11932–11935
30. Georgopoulos, K., Winandy, S., and Avital, N. (1997) *Annu. Rev. Immunol.* 15, 155–176
31. Morgan, B., Sun, L., Avital, N., Andrikopoulos, K., Ikeda, T., Gonzalez, E., Wu, P., Neben, S., and Georgopoulos, K. (1997) *EMBO J.* 16, 2004–2013
32. Merika, M., and Orkin, S. H. (1995) *Mol. Cell. Biol.* 15, 2477–2477
33. Engert, C., Vidal, M., Maheswaran, S., Ge, Y., Ezzell, R. M., Isselbacher, K. J., and Haber, D. A. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 11960–11964
34. Moffett, P., Bruening, W., Nakagama, H., Bardeesy, N., Housman, D., Housman, D. E., and Pelletier, J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 11105–11109
35. Nachitgal, M. W., Horiokawa, Y., Eyenveart-Van Houten, D. L., Flanagan, J. N., Hammer, G. D., and Ingraham, H. A. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 93, 445–454
36. Wang, Z. Y., Qiu, Q. Q., Seufert, W., Taguchi, T., Testa, J. R., Whitmore, S. A., Calhoun, D. F., Walsh, D., Shenk, T., and Deuel, T. F. (1996) *J. Biol. Chem.* 271, 24811–24816
37. Maheswaran, S., Engert, C., Zheng, G., Lee, S. B., Wong, J., Harpark, D. P., Bean, J., Ezzell, R., Garvin, A. J., McCluskey, R. T., DeCaprio, J. A., and Haber, D. A. (1998) *Genes Dev.* 12, 1168–1172
38. Sekiya, M., Adachi, M., Hinoda, Y., Imai, K., and Yachi, A. (1994) *Blood* 83, 1867–1882
39. Baird, P. N., and Simmons, P. J. (1997) *Exp. Hematol.* 25, 312–320
40. Maurer, U., Briege, J., Weidmann, E., Mitrou, P. S., Hoezer, D., and Bergmann, L. (1997) *Exp. Hematol.* 25, 945–960
41. Messen, H. D., Renkli, H. J., Entexami, M., and Thiel, E. (1997) *Blood* 89, 3486–3487
42. Inoue, K., Sugiyama, H., Ogawa, H., Nakagawa, M., Yamagami, T., Miwa, H., Kita, K., Hiraoka, A., Masaoka, T., and Nasu, K. (1994) *Blood* 84, 3071–3079
43. Pritchard-Jones, K., and King-Underwood, L. (1997) *Leuk. & Lymphoma* 27, 207–220
44. Ellisson, L. W., Carlesso, N., Cheng, T., Scadden, D. T., and Haber, D. A. (2001) *Proc. Natl. Acad. Sci. U. S. A.* 98, 1807–1909
45. Smith, S. I., Down, M., Boyd, A. W., and Lé, C. L. (2000) *Cancer Res.* 60, 808–814
Inhibition of Wilms Tumor 1 Transactivation by Bone Marrow Zinc Finger 2, a Novel Transcriptional Repressor
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