KAISO, a critical regulator of p53-mediated transcription of CDKN1A and apoptotic genes

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An unresolved issue in genotoxic stress response is identification of induced regulatory proteins and how these activate tumor suppressor p53 to determine appropriate cell responses. Transcription factor KAISO was previously described to repress transcription following binding to methylated DNA. In this study, we show that KAISO is induced by DNA damage in p53-expressing cells and then interacts with the p53–p300 complex to increase acetylation of p53 K320 and K382 residues, although decreasing K381 acetylation. Moreover, the p53 with this particular acetylation pattern shows increased DNA binding and potently induces cell cycle arrest and apoptosis by activating transcription of CDKN1A (cyclin-dependent kinase inhibitor 1) and various apoptotic genes. Analogously, in Kaiso KO mouse embryonic fibroblast cells, p53-to-promoter binding and up-regulation of p21 and apoptosis gene expression is significantly compromised. KAISO may therefore be a critical regulator of p53-mediated cell cycle arrest and apoptosis in response to various genotoxic stresses in mammalian cells.

KAISO | p53 | cell cycle arrest | apoptosis | p300

The POZ/BTB family protein KAISO was first isolated based on its interaction with the cell adhesion catenin, p120ctn, a protein with structural similarity to the beta-catenin of the Wnt signaling pathway (1, 2). KAISO has been described as a methyl CpG-binding domain (MBD) protein, and binds to both a sequence-specific KAISO binding site and methylated CpG dinucleotides within target gene promoters (3–5). KAISO has been shown to interact with the nuclear receptor corepressor (NCoR) and mediate DNA methyltransferase-dependent transcriptional repression (4, 6, 7). It has also been suggested that KAISO is a transcription factor that regulates the cell cycle and is a potential tumor suppressor able to block cancer progression (2, 8). However, when crossed with intestinal and mammary tumor-susceptible ApcMin/+ mice, Kaiso-null mice showed a delayed onset of intestinal tumorigenesis, suggesting that Kaiso deficiency decreases tumor size and initiation (9). Likewise, Kaiso has been shown to be up-regulated in murine intestinal tumors and is expressed in human colon cancers (9), suggesting that Kaiso may be an oncogene that induces intestinal cancer. Thus, the role of KAISO as either a tumor suppressor or an oncogene remains ambiguous.

The tumor suppressor p53 is a key mediator of cellular responses to a variety of stresses (10). It is activated in response to DNA damage and mediates cell cycle arrest, DNA repair, senescence, and apoptosis (10, 11). How p53 is able to discriminate between different stresses and elicit specific cellular response is important and intriguing, but remains largely elusive. When cells are exposed to stresses such as DNA damage, transcriptional outcomes can be determined by the sequence of p53-responsive DNA element (p53RE), levels of induced p53, posttranslational modifications of p53, p53-interacting proteins, and the epigenetic landscape of p53 target gene promoter. Posttranslational acetylation of p53 by acetyltransferases has been found to be important in cell cycle arrest and/or apoptosis by increasing p53 DNA binding and transcription activation potential (12–14). More specifically, how p53 is activated by a particular signal and how it reads a specific p53RE to regulate transcription, of distinct target genes, remains largely unresolved. These questions are important, as the transcriptional output of p53 target genes regulates cell fate decisions in response to a specific genotoxic stress.

Apoptosis, an essential process in mammalian growth and development and cancer, can be induced by cancer therapeutic agents, hypoxia, mitotic abnormalities, and ionizing irradiation. In mammals, there are two major apoptosis pathways (15). The intrinsic apoptosis pathway is regulated by p53 and proapoptotic proteins of the B-cell lymphoma 2 (BCL-2) family of proteins, including p53 up-regulated modulator of apoptosis (PUMA), phosphol-12-myristate-13-acetate-induced protein 1 (NOXA), Bcl-2-associated X protein (BAX), and BH3-interacting domain death agonist (BID). PUMA, a major player in apoptosis, seems to be exclusively controlled by transcription and p53 is indispensable for its transcriptional activation (16, 17). The extrinsic apoptosis pathway is induced by “death ligands” that bind to death receptors, including FAS (CD95 or apoptosis antigen 1 [APO-1]), death receptor 5 (DR5), and p53 apoptosis effector related to PMP-22 (PERP). Activation of the apoptosis pathway releases cytochrome C from the mitochondria and activates caspase 9 and the effector caspases 3, 6, and 7 that finalize the apoptosis process (15, 18). Expression of death receptor genes and apoptotic protease-activating factor 1 (APAF-1) is also controlled by p53 (19, 20).

In this study, we found that KAISO expression is increased by etoposide treatment in a p53-dependent manner and forms a complex with p53 and the acetyltransferase p300. Subsequently, we

Significance

Transcription factor KAISO (POZ/BTB family protein, ZBTB33) expression is induced by genotoxic stress in a tumor suppressor p53-dependent manner. KAISO then interacts with p53 and the acetyltransferase p300 to modulate p300 acetylation of p53 and increasing upon p53 a “code,” i.e., acetylation at K320 and K382, and inhibition of acetylation at K381. This coded p53 shows increased DNA binding to p53 response elements in the promoters of CDKN1A (cyclin-dependent kinase inhibitor 1) and apoptosis genes, subsequently inducing cell cycle arrest and potent apoptosis. KAISO is a critical regulator of DNA damage responses in multiple cell types and carries out this function by regulating p53-mediated cell cycle arrest and apoptosis.

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comprehensively investigated the functions of KAISO in response to DNA damage and the molecular mechanisms underpinning cell cycle arrest and apoptosis induced by a KAISO–p53–p300 interaction.

**Results**

**KAISO Induces Cell Cycle Arrest and Apoptosis and Forms a Protein Complex with p53 and p300.** Recently, we and others found that some BTB/POZ-domain proteins with Krüppel-like zinc-fingers (POK family) function either as tumor suppressors or as oncoproteins (21). We investigated which POK transcription factors are induced in response to DNA damage and could serve as tumor suppressors. Human HEK293 cells were exposed to the DNA-damaging agent etoposide and the expression of various POK transcription factors analyzed. We found that KAISO, p21, p53, BAX, and PUMA were increased both at the mRNA and protein levels (SI Appendix, Fig. S1A). We further explored whether KAISO could regulate the p53-controlled cell cycle arrest and apoptosis. Analysis of cell proliferation by direct cell number counting or MIT cell viability assay and flow cytometry showed that ectopic KAISO inhibited cell proliferation and increased the number of cells undergoing apoptosis (Fig. 1 and SI Appendix, Fig. S1 B–D). In contrast, a KAISO K8 mutant, lacking the POZ domain and zinc-finger functional domains, showed attenuated apoptotic activity and an inability to induce apoptosis genes (SI Appendix, Fig. S2). Activation and posttranslational modification of p53 by acetyltransferases (p300, PCAF, Tip60) are known to be key regulators of p53-mediated apoptosis (12, 22–24). Consequently, we investigated whether p53 or acetyltransferases interact with endogenous KAISO under normal and etoposide-treated conditions. We found that p53 and KAISO interact with each other under both conditions; more protein complex, however, was formed following etoposide treatment. KAISO also interacted with p300 and Tip60, but not PCAF, under normal condition. Whereas the level of the complex of KAISO and Tip60 was not altered, KAISO/p300 complex levels increased following etoposide treatment (Fig. 1B).

We also investigated whether these proteins interact directly with each other and mapped their interacting domains. Coimmunoprecipitation and GST-fusion pull-down assays showed that KAISO, p53, and p300 interact with each other to form a heterotrimeric complex (Fig. 1C and SI Appendix, Fig. S3). KAISO interacted with the C-terminal DNA-binding domain of p53 via its POZ domain and zinc-finger DNA-binding domain (ZFDBD). The POZ domain interacted directly with the DUF906 domain (no. 3) of p300, encompassing its acetyltransferase enzymatic region. These data suggest that KAISO may regulate cell cycle arrest and apoptosis by affecting acetylation of p53 by p300 through p53–p300–KAISO complex formation.

**KAISO Influences the Ability of p300 to Acetylate the Three Lysine Residues of p53 and Confers p53 a KAISO-Specific Acetylation Code.** We next investigated possible influence of KAISO on p53 acetylation by p300. Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of an in vitro p53 acetylation reaction mixture (absent KAISO) revealed that lysine residues of p53 reported to be acetylated by p300 (K164, K305, K370, K372, K373, K381, K382, and K386) could be acetylated (SI Appendix, Fig. S4 I–L). Interestingly, addition of KAISO to the above reaction mixture affected acetylation of three lysine residues of p53 as follows: increased acetylation of K382, acetylation of K320 (previously reported not to be acetylated by p300), and inhibition of acetylation of K381 (Fig. 2 and SI Appendix, Fig. S4 B–H). For K320, although we were unable to obtain a clean extracted ion chromatogram (XIC), we observed a mass spectrum corresponding to an acetylated p53 polypeptide fragment (amino acids 320–333, K(Ac)KPLDGELYTFLQIR) only in the p53–KAISO–p300 reaction mixture (Fig. 2A). K381, a residue previously known as one of the target sites of acetylation, ubiquitination, and p53 degradation, was blocked from acetylation following its interaction with KAISO (Fig. 2 B and C). Western blot assays of HEK293 cell lysates with ectopic or knocked-down KAISO, using anti–Ac-K320–, anti–Ac-K381–, and anti–Ac-K382–specific antibodies, revealed that KAISO promoted acetylation of p53 at K320 and K382, but inhibited acetylation of K381 (SI Appendix, Fig. S4 M and N). These results are consistent with our LC-MS/MS data described above.

**Molecular Interactions Between KAISO, p53, and p300 Increase p53 DNA Binding to p53REs in CDKN1A and Apoptotic Genes.** Because KAISO induces cell cycle arrest and apoptosis, we investigated whether and how KAISO might regulate expression of CDKN1A, PUMA, BAX, and p53. p21 and PUMA, which represent the major regulators of cell cycle arrest and apoptosis, are directly regulated at the transcriptional level by p53. Whereas ectopic KAISO increased p21, PUMA, and BAX expression, KAISO knockdown decreased their expression in HEK293 cells (Fig. 3A). KAISO did not affect p53 expression. Thus, KAISO may directly bind p53 response elements or otherwise affect p53 activity to activate expression of p53 target genes. Oligonucleotide pull-down assays revealed that KAISO alone (absent p53) lacks the ability to bind the p53RE of CDKN1A and PUMA (compare binding patterns in p53**+/−** vs. p53**+/−**/HCT116 human colon cancer cells transfected with KAISO expression vector (Fig. 3B).**

![Fig. 1. KAISO induces apoptosis and interacts with the p53–p300 complex. (A) Western blot of KAISO overexpression in HEK293 cells transfected with KAISO vector (Left). Bar graph showing quantification of flow cytometry analysis of apoptosis of HEK293 cells transfected with KAISO expression vector (scatterplots shown on Right). *P < 0.005. (B) Coimmunoprecipitation and Western blots of endogenous KAISO, p53, and histone acetyltransferase proteins. HEK293 cells were treated with vehicle or etoposide and the cell lysates prepared 24 h posttreatment. (C) Coimmunoprecipitation and Western blots showing the various interactions among p53, KAISO, and p300. HEK293 cells were transfected with a KAISO expression vector and cell lysates immunoprecipitated with anti-p300, anti-p53, or anti-KAISO antibodies.](image-url)
Oligonucleotide pull-down assays of the in vitro p53 acetylation reaction mixture p53 + p300 + KAISO showed that p53 binding to p53RE1 of CDKN1A and PUMA was significantly increased by KAISO (SI Appendix, Fig. S5). ChIP assays showed that KAISO increased DNA binding of p53 and p50 in the p53REs of the CDKN1A and PUMA promoters (Fig. 3C). ChIP-ReChIP assays also showed that ectopic p53WT and KAISO bind together to the p53REs of the promoters of CDKN1A, PUMA, and other apoptosis genes, in H1299 p53-null human lung cancer cells (Fig. 3D and SI Appendix, Fig. S6 C and D). Additional ChIP assays of p53 binding in Kiso WT and KO mouse embryonic fibroblasts (MEFs) treated with etoposide showed that p53 binding to CDkn1a and apoptosis gene promoter regions flanking the p53REs increased only in the presence of Kaiso (Fig. 3E and SI Appendix, Fig. S6F). We also found that KAISO binding to CDKN1A and apoptosis gene promoters required p53 in the HCT116 p53+/− and p53−/− cells treated with etoposide (Fig. 3F and SI Appendix, Fig. S6G).

These data suggest that the in vivo molecular interactions between KAISO, p53, and p300 regulate the combinatorial acetylation of the three aforementioned lysine residues of p53, and that these events are important in increasing p53 DNA binding and transcriptional activation of p53 target genes such as CDKN1A, PUMA, and other apoptotic genes.

Intriguingly, the p53REs of CDKN1A and apoptotic genes regulated by KAISO showed a bipartite symmetrical arrangement of their half sites (RRRCWWGGYYY). These two half sites face each other with a 0- or 1-nucleotide spacer between them (→← spacer →←). In contrast, KAISO did not enhance p53-mediated repression of the antiapoptosis gene Survivin or activation of the DDA3 gene at the transcriptional level (SI Appendix, Fig. S6C). The latter two genes have two half sites in a similar orientation separated by 3- and 9-nucleotide spacers, respectively (SI Appendix, Table S2).

Introduction of an extra 5-nucleotide spacer between the two half sites of p53REs of CDKN1A and PUMA significantly decreased p53 binding in a p53 + p300 + KAISO mixture, but not in a p53 + p300 (lacking KAISO) mixture or p53 alone (SI Appendix, Fig. S5). These results suggest that combinatorial modification of the three p53 lysine residues may induce a conformational change such that it can more efficiently bind two p53RE half sites with 0- or 1-nucleotide spacers.

The Acetylation Pattern of the Three p53 Lysines Affected by KAISO/ p300 Is Critical for Transcriptional Activation of p53 Target Genes, CDKN1A, and Apoptotic Genes. To study possible functions of KAISO/p300-associated p53 acetylation in apoptosis, we used site-directed mutagenesis to prepare p53 mutants representing the above-described p53 code: an acetyl mimetic, p53RQR (K320Q, K381R, K382Q), and a functionally negative form of p53QOR (K320R, K381Q, K382K). Transient transfection assays in H1299 cells showed that ectopic p53 could activate transcription of endogenous CDKN1A and apoptotic genes. Additionally, when KAISO and p53 were coexpressed, they further stimulated the expression of apoptosis genes. Similarly, the p53RQR mutant potently activated transcription of the same genes, in contrast to the functionally negative form, p53QOR (SI Appendix, Fig. S7A). Under these test conditions, the expression levels of p53WT, p53QOR, and p53QOR were all similar, at both the mRNA and protein levels (SI Appendix, Fig. S7 C and D).

We further used flow cytometry to demonstrate that p53QOR could induce apoptosis in H1299 cells, comparable to that seen following coexpression of KAISO and p53 (Fig. 4). In contrast, the functionally negative p53RQR mutant showed no significant effect on apoptosis. Moreover, p53 could not induce apoptosis when KAISO expression was knocked down by siRNA, suggesting that KAISO is essential for this p53 function (Fig. 4 and SI Appendix, Fig. S7 B and E). These data collectively suggest that the combinatorial acetylation of three p53 lysine residues by p300 (imposed by KAISO) is important and sufficient for transcriptional activation of a group of p53 target genes that regulate apoptosis.

KAISO Plays an Indispensable Role in Cdkn1a and Apoptotic Gene Expression in MEF Cells. We also investigated the role of KAISO in cell cycle arrest and apoptosis using a loss-of-function approach. The level of p53 expression was nearly identical in MEF cells with both Kiso WT and KO phenotypes, but the expression of the p53 target genes Cdkn1a, Bax, Puma, death receptor genes (Fas and Dr5) of the extrinsic pathway (9, 25, 26), and caspase genes was low or not detected in Kiso KO MEF cells (Fig. 5A and SI Appendix, Fig. S8 C and D). Whereas etoposide and the death receptor ligands FasL and Trail induced expression of Kiso, p53 and other genes important in cell cycle arrest and apoptosis (Cdkn1a, Bax, Puma, Caspase 8, Caspase 3, Dr5, and Fas) in Kiso WT MEF cells, they did not induce expression of these genes in Kiso KO MEF cells, even when p53 was induced (Fig. 5B and SI Appendix, Fig. S8 C and D). Interestingly, expression of two critical players in apoptosis, Caspase 8 and Caspase 3 was also significantly decreased in the absence of Kiso, which may also reflect a decrease in the expression of death receptors and apoptotic protease-activating factor 1 (Apaf-1) (SI Appendix, Fig. S8 C and D). RT-qPCR analysis indicated that mRNA expression of Cdkn1a and apoptosis pathway genes was very low or not detectable in Kiso KO MEF cells. Even when Kiso KO MEF cells were treated with etoposide, all of the genes were not induced, although p53 mRNA induction was similar to that in WT MEF cells (Fig. 5C). We also noticed that death receptor ligands could activate KAISO expression (SI Appendix, Fig. S8 C and D), and that KAISO
mRNA knockdown attenuated apoptosis of HCT116 cells by the death receptor ligand Trail (SI Appendix, Fig. S8 A and B).

We also investigated whether RNA polymerase II (Pol II) binding during transcription of apoptotic genes, Kaiso, and transformation-related protein 53 (Trp53) was affected by the presence or absence of Kaiso in MEFs exposed to etoposide. ChIP assays of RNA polymerase II binding to exonic regions (thus detecting elongating RNA Pol II) showed that RNA Pol II binding to Kaiso and apoptotic genes significantly decreased following etoposide treatment in Kaiso KO MEFs. In contrast, RNA Pol II binding to the downstream exons of Trp53 was increased by etoposide in both Kaiso WT and KO MEFs. Furthermore, ChIP assays of RNA Pol II involved in transcriptional initiation, elongation, and termination, using RNA Pol II antibodies (CTD4H8, Ser2Ph, and Ser5Ph), showed that expression of Kaiso and/or p53 increases RNA Pol II in elongation and termination stages and transcription of PUMA and BAX (Fig. 5D and SI Appendix, Fig. S9 A-D). These results suggest that Kaiso plays an important role in the transcriptional activation of p53 target apoptosis genes.

Kaiso Inhibits Proliferation of MEFs and Plays a Critical Role in Apoptosis. We showed that chemotherapeutic agents, ionizing radiation, and UV radiation, known stressors that induce apoptosis via p53 expression, could activate Kaiso expression (SI Appendix, Fig. S10 A and B), which would eventually affect activity of p53 and the expression of apoptosis genes. Furthermore, we analyzed cell growth rates of Kaiso WT and KO MEFs after pretreatment with etoposide. Kaiso WT MEFs underwent a severe decrease in cell number at 1–4 d postetoposide treatment, whereas Kaiso KO MEFs showed mild decrease in cell number and were relatively etoposide resistant (Fig. 6A). Terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assays revealed fewer apoptotic cells among Kaiso KO MEFs compared with Kaiso WT MEFs. The ratios of apoptotic cells to the total number of 4', 6-diamidino-2-phenylindole (DAPI)-positive cells were 44% (SD ± 2.6%) for
Kaiso WT and 2.24% (SD ± 1.0%) for Kaiso KO MEFs, respectively (Fig. 6B). KAIOSO thus induces potent apoptosis in the WT MEF cells exposed to etoposide.

Microarray analyses of spleen and testis tissues of Kaiso WT and KO mice demonstrated that Puma, Noxa, Dr5, and FasL mRNA were significantly decreased in KO mouse tissues (SI Appendix, Table S1). In the brain, kidney, and testis tissues of C57BL/6J Kaiso KO mice (9), p53 expression levels were nearly identical to those in Kaiso WT mice; however, the expression levels of Puma were markedly decreased at both the mRNA and protein levels (SI Appendix, Fig. S10 C and D). Immunohistochemical (IHC) analysis of testis tissues showed that the expression of Bax decreased significantly in the absence of Kaiso (SI Appendix, Fig. S10F). The number of cells positive for proliferating cell nuclear antigen (Pena) in Kaiso knockout mouse tissues was high, compared with wild-type controls. Also, MEF KO cells prepared from the Kaiso KO mouse embryos were morphologically similar to MEF WT cells but grew 2.5 times faster after 5 d (Fig. 6C). These results show that KAIOSO inhibits cell proliferation and plays a role in apoptosis.

Discussion

One of the interesting questions surrounding p53-mediated transcription is how p53 specifically recognizes only certain subsets of promoters and ignores numerous other promoters in response to different stimuli (27). p53-binding site sequences, p53 postranslational modifications (23, 28), and p53-interacting proteins are important determinants of the diversity of p53-mediated transcriptional responses. For example, increased DNA binding and stability have been demonstrated following acetylation of p53 lysine residues K120, K164, and K320 by Tip60/HDAC; p300/CBP, and P300/CBP-mediated acetylation of lysine residues K370, K372, K373, K381, K382, and K386 in the p53 C terminus (13, 25, 26, 28–32). Each of these findings highlights the importance of modification of specific lysine residues, but the combined effect of multiple p53 modifications on gene expression is largely unknown.

The p53 protein interacts with coactivators or corepressors and can be acetylated or deacetylated to respectively activate or repress its transcriptional activity. Proteins interacting with p53 can modulate acetylation levels by altering the acetyltransferase or deacetylase activity of coregulators, thus affecting promoter DNA binding by p53 and transcription activation potential (23, 28–32). We demonstrated that expression of KAISO is induced by etoposide in the cells expressing p53 and forms a heterotrimeric complex with p53 and p300. KAISO also affects p300 acetylation of three key lysine residues of p53, increasing acetylation at K320 and K382, while inhibiting acetylation of K381. We also showed that p53 acetylation pattern following its interaction with KAISO-p300, could similarly activate transcription of CDKNTA and apoptotic genes and thereby induce cell cycle arrest and apoptosis (refer to model shown in Fig. 6D). Levine and Oren (27) and Levine and coworkers (33) discussed the influence of p53 posttranslational modifications, p53RE arrangements, and spacer lengths, and p53RE nucleotide sequences on the activation of p53 and its transcriptional activity.
downstream target genes. How does KAISO activate transcription of a group of genes that plays roles in cell cycle arrest and apoptosis? The p53RE of CDKN1A and apoptosis genes regulated by KAISO showed a bipartite symmetrical arrangement of p53RE half sites (RKKRCWWGYYY) with a 0- or 1-nucleotide spacer. In contrast, KAISO did not enhance transcriptional regulation of genes having the p53RE with 3- or 9-nucleotide spacers (SI Appendix, Table S2 and Fig. S6C). The combined effect of modification of the three p53 lysine residues may be induction of a conformational change that can more efficiently bind the two half sites of the p53RE separated by 0- or 1-nucleotide spacers.

**Kaiso KO C57BL/6J mice** maintained in the heterogeneous genetic backgrounds Ola120 and C3H were viable and fertile, and they exhibited no detectable abnormalities of development or gene expression (9). Kaiso-deficient mice, in the absence of the tumor suppressor APC, were susceptible to intestinal cancer, suggesting that Kaiso might be an oncogene (9). By contrast, KAISO has also been suggested to be a potential tumor suppressor repressing transcription of MMP7, Cyclin D1, and Wnt11, genes involved in oncogenesis and metastasis (1, 2, 34). However, Kaiso KO mice maintained in a C57BL/6J genetic background for three generations showed several distinct phenotypes such as splenomegaly, testis atrophy, and increased body weight and size. This failure to observe overt phenotypes in previous studies and in our Kaiso KO G1 and G2 generation mice may indicate a role for Kaiso as a methylcytosine-binding domain (MBD) family protein involved in the epigenetic regulation of target genes. Such regulation could be maintained for three generations after Kaiso knockout or may be due to differences in the genetic backgrounds in which Kaiso gene function was tested. Microarray analysis of differentially expressed mRNA in mouse tissues showed massive changes in mRNA expression of as many as 4,000 genes. Our investigation consistently revealed that KAISO plays an important role in cell cycle arrest and apoptosis in HEK293, H1299, and MEF cells and Kaiso KO mice. Expression of Cdk11a, Puma, and Bax genes was significantly decreased in Kaiso-null MEFs and in various tissues of Kaiso KO mice. MAEKO may be a potential tumor suppressor inducing cell cycle arrest and apoptosis. Interestingly, HCT116 cells transfected with KAISO siRNA showed an increase in apoptosis when treated with etoposide (7), suggesting that KAISO is antipapotic. In contrast, we found that KAISO was induced by etoposide robustly enough to overcome or rescue knocked down KAISO expression in both HCT116 and HEK293 cells (SI Appendix, Fig. S10 F and G). Our similar experiment carried out with Kaiso KO MEFs clearly showed that etoposide treatment induced little apoptosis (Fig. 6A), further suggesting that KAISO is proapoptotic.

We also found that other cellular stresses, including UV radiation, γ-irradiation, chemotherapeutic anticancer drugs, and death receptor ligands also induced KAISO expression. These agents may induce apoptosis through KAISO, similar to etoposide. In summary, our study exemplifies the importance of KAISO to p53-mediated transcription of CDKN1A and apoptosis genes.

**Materials and Methods**

**Kaiso KO Mice.** Animal experiments were approved by the Committee on Animal Investigations of Yonsei University.

**LC-MS/MS.** Mass spectrometry, in-gel tryptic digestion, and on-line nano LC-MS/MS were performed as previously reported (35, 36).

**ChIP/Re-ChIP Analysis.** ChIP/Re-ChIP assays were performed to assess KAISO, p53, and p300 binding to the promoters of apoptotic genes in vivo, and the sequences of the oligonucleotide primers used in these assays are listed in SI Appendix, SI Materials and Methods.

**In Vitro Protein Acetylation Assays.** KAISO protein was incubated with p300 and His-p53 in vitro as p53 acetylation assay buffer for 1 h at 37 °C, and the mixture was then resolved by SDS/PAGE, stained using Coomassie Blue, and gel regions containing p53 were excised and analyzed by mass spectrometry. The full methods are available in SI Appendix, SI Materials and Methods.

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