AKR1B10, a Transcriptional Target of p53, Is Downregulated in Colorectal Cancers Associated with Poor Prognosis

Tomoko Ohashi¹, Masashi Idogawa¹, Yasushi Sasaki¹, Hiromu Suzuki², and Takashi Tokino¹

Abstract

p53 is one of the most important tumor suppressor genes, and it is frequently inactivated in various cancers. p53 modulates various cellular functions, such as apoptosis and cell-cycle arrest via transcriptional regulation. Recently, p53 has been reported to be involved in a wide range of cellular metabolic pathways, including glycolysis, oxidative phosphorylation, glutaminolysis, and the antioxidant response. To understand the functional mechanism of p53, it is important to find out the direct transcriptional targets of p53. In this study, aldo-keto reductase family 1, member B10 (AKR1B10) was identified as a direct target of the p53 family by cDNA microarray analysis after comparing the mRNA expression of control and H1299 cells that overexpressed with p53 family members. In addition, we found that the expression of AKR1B10 was significantly decreased in colorectal cancers and adenomas as compared with normal colon tissues. Knockdown of AKR1B10 significantly inhibited p53-induced apoptosis in colorectal cancer cells, whereas the overexpression of AKR1B10 enhanced p53-induced apoptosis and inhibited tumor proliferation in vivo. Furthermore, low expression of AKR1B10 in colon cancer patients was correlated with decreased survival and poor prognosis. These results suggest that decreased expression of AKR1B10 could disrupt the tumor suppressive function of p53, which result in decreased survival in colorectal cancer patients. In summary, AKR1B10 may be a novel prognostic predictor and a novel therapeutic target for colorectal cancer.

Implications: AKR1B10, a transcriptional target of p53, is also a novel prognostic and therapeutic molecule in colorectal cancer. Mol Cancer Res; 11(12); 1554–63. ©2013 AACR.

Introduction

p53 is one of the most important tumor suppressor genes and is frequently inactivated in gastrointestinal cancers, such as colorectal cancers, esophageal cancers, and gastric cancers, as a direct result of mutations in the p53 gene (1, 2). Furthermore, mutation or deletion of p53 is related to poor prognosis and resistance to chemotherapy and radiation (3). The p53 protein is activated by a variety of cell stresses, such as DNA damage, oncogene activation, spindle damage, and hypoxia, and activated p53 transactivates a number of target genes, many of which are involved in DNA repair, cell-cycle arrest and apoptosis (4). Recently, p53 has been reported to be involved in a wide range of cellular metabolic pathways, including glycolysis, oxidative phosphorylation, glutaminolysis, and the antioxidant response (5, 6). In addition, other p53 family members, such as p63 and p73, also induce cell-cycle arrest and apoptosis and play an important role in development and differentiation (7), and dominant negative forms of p63 and p73 are overexpressed in some types of cancers (8, 9). For example, at least 30% of head and neck squamous cell carcinomas harbor mutations in genes that regulate squamous differentiation, including p63 (10). Therefore, many studies have sought to identify target genes of p53 and its family members because these proteins execute diverse functions, mainly through transcriptional regulation.

To identify direct targets of p53 family members, we compared mRNA expression in H1299 cells overexpressing p53 family members and control cells through cDNA microarray and identified aldo-keto reductase family 1, member B10 (AKR1B10) as a direct target of the p53 family. Interestingly, knockdown of AKR1B10 significantly inhibited p53-induced apoptosis in colorectal cancer cells, whereas overexpression of AKR1B10 enhanced p53-induced apoptosis and inhibited tumor proliferation.

Furthermore, we found that the expression of AKR1B10 was significantly decreased in human colorectal cancers and adenomas and that the rates of disease-free or overall survival were significantly lower among patients whose tumors had low levels of AKR1B10 expression compared with those with high levels of AKR1B10 expression.
AKR1B10, a Target of p53, is Downregulated in Colon Cancers

Materials and Methods

Cell culture

H1299 human lung cancer cells, SaOS-2 osteosarcoma cells and RKO, LoVo, BM314, DLD1, and SW480 colon cancer cells were purchased from the American Type Culture Collection and the Japan Collection of Research Bioresources. Colon cancer HCT116 (p53-/-) and the derivative HCT116 (p53+/+) cell line were kindly provided by Dr. B. Vogelstein (Howard Hughes Medical Institute, Johns Hopkins University, Baltimore, MD). The construction, purification, and infection of replication-deficient recombinant adenoviruses containing FLAG-tagged p53 (Ad-p53), TAp63 (Ad-p63), and TAp73 (Ad-p73) or the bacterial lacZ gene (Ad-LacZ) have been described previously (11).

Chromatin immunoprecipitation

H1299 cells were infected with Ad-FLAG-p53, p63, p73, and LacZ at a multiplicity of infection (MOI) of 25. At 24 hours after infection, these cells were subjected to chromatin immunoprecipitation (ChIP) with an anti-FLAG antibody or normal mouse IgG as a control using the ChIP Assay Kit (Upstate) according to the manufacturer's protocol. HCT116 (p53+/+) cells were treated with adriamycin (0.5 μg/mL). At 24 hours after treatment, these cells were subjected to ChIP with anti-p53 (DO-7) antibody (Santa Cruz). ChIP-sequencing (ChIP-seq) data were deposited to the DDBJ sequence read archive (DRA, accession number: DRA000614) in a previous study. Primer sequences for PCR were ACCCCTACCCGGAGGTGAAAA and TGGAGGTGTTCAATCCGGACCT for AKR1B10/RE1 and GCCCTGTCATTTTGTGTTGGGAA and GATTACCTGGCATGACAGTACAGGAC for AKR1B10/RE2.

Antibodies and reagents

Adriamycin and flufenamic acid were purchased from Sigma. The anti-FLAG (M2) mouse antibody was purchased from Sigma; the anti-FLAG rabbit antibody was purchased from R&D Systems; the anti-FLAG mouse antibody was purchased from Millipore; and the anti-PARP-1 mouse antibody was purchased from BD Pharmingen.

Western blot analysis

Total cell lysate was extracted at 4°C with RIPA buffer (150 mmol/L NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mmol/L Tris-HCl, pH 8.0). The samples were fractionated by SDS-PAGE and transferred onto Immobilon-P membranes (Millipore). Immunoreactive proteins were detected using enhanced chemiluminescence (ECL; GE Healthcare).

Reverse transcription (RT)-PCR

Total RNA was prepared from cell lines using the RNeasy Mini kit (QIAGEN). For RT-PCR analysis, cDNA was synthesized from 5 μg of total RNA using SuperScript III (Life Technologies). GoTaq (Promega) was used for PCR and quantitative PCR (qPCR). For qPCR experiments, TaqMan probes (Applied Biosystems) were used (AKR1B10: Hs01546975_gH, CDKN1A: Hs00355782_m1, GAPDH: Hs99999905_m1) and the mean value of 3 replicates was normalized using GAPDH.

Luciferase reporter assay

AKR1B10-RE1 (tGGCcTGTCa tGGGgTGTCa), RE1 mutant (tGGacGT>Tc aGGGgTTcTc), or RE2 (ttcAgGT-TT GGGCAGTTCa), RE2 mutant (ttcAgGT>TTC GGGAATTCa) ± 10 bp was subcloned into the pGL3-promoter plasmid (Promega). Cells were transiently transfected in triplicate using a luciferase reporter, pHRG-TK (Promega) and expression vectors for p53, p63γ, p73β, or MOCK inserted into pCMV-Tag2-FLAG (Stratagene) using Lipofectamine 2000 (Life Technologies). Luciferase activity was measured with the Dual-Luciferase Reporter Assay System (Promega). Renilla luciferase activity was used as an internal control.

siRNAs

The cocktail of 4 siRNAs targeting AKR1B10 (M-009691-01) was purchased from Thermo. A nontargeting siRNA pool was also purchased as a negative control from Thermo.

Flow cytometry

For flow cytometry, 1 × 10⁶ cells were plated in 60-mm plates. At 24 hours after plating, cells were transfected with siRNA using Lipofectamine RNAiMAX (Life Technologies). At 4 hours after transfection, cells were infected with adenovirus, and at 24 hours after infection, the cells were again transfected with siRNA. The cells were analyzed using a FACSCalibur flow cytometer (BD Bioscience) at 48 hours after infection, as previously described (12). Experiments were repeated at least 3 times, and 5 × 10⁶ events were analyzed for each sample. The resulting data were analyzed using FlowJo software (TreeStar).

Determination of caspase activity

Caspase activity was evaluated using a caspase-3, -8, and -9 colorimetric assay kit (Biovision), according to the manufacturer's instructions. This kit utilizes synthetic tetrapeptides labeled with p-nitroanilide. Briefly, cells were lysed in the lysis buffer supplied with the kit, and the supernatants were collected and incubated at 37°C with reaction buffer containing dithiothreitol and substrates. Caspase activity was determined by measuring the changes in absorbance at 405 nm using a microplate reader.

Microarray analysis

Total RNA was labeled with Cy3, hybridized to a microarray (Agilent SurePrint G3 Human GE, including probes corresponding to 27,958 annotated transcripts), and scanned by Agilent SureScan, according to the manufacturer's
protocols. The obtained data were normalized using Limma (R package). The microarray data were deposited to the NCBI Gene Expression Omnibus (GEO, accession number: GSE47096). Genes with greater than 2-fold changes in expression in comparison to control cells were selected for further analysis. The expression levels were converted to Z-scores and subjected to hierarchical clustering based on the average Euclidean distance using gplots (R package).

Animal models
All animals were maintained under specific pathogen-free conditions and treated in accordance with the guidelines set by the Animal Care and Use Committee of Sapporo Medical University. To evaluate the effect of treating established tumors, 8 female BALB/c nude mice were injected subcutaneously into both flanks with \(2 \times 10^6\) LoVo or DLD1 cells. The tumor volume was calculated using the equation \(V = a \times b^2 / 2\), where “a” is the largest dimension and “b” is the perpendicular diameter.

Analysis of gene expression datasets
Four gene expression datasets of human colorectal cancers (GEO: GSE20916, GSE3964, GSE9348, and GSE5261; refs. 13–16) and 2 gene expression datasets of human colorectal adenomas (GEO: GSE20916 and GSE9689; refs. 16 and 17) were analyzed in Oncomine, a cancer microarray database (www.oncomine.org/). In 3 gene expression datasets of human colorectal cancers including survival information (GEO: GSE17536, GSE12945, and GSE14333; refs. 18–20), patients were divided into 2 groups: those with high and low expression of AKR1B10 according to the Prognostic algorithm (21). The survival curve was constructed using the Kaplan–Meier method and survfit (R package). P values were calculated by log-rank test using survdiff (R package).

Results
The transactivation of AKR1B10 by p53 family members
We first infected human lung cancer H1299 cells (p53-null) with adenoviral vectors expressing p53 family members or LacZ as a control and compared mRNA expression using cDNA microarray. We identified 223 genes, the expression of which was increased by p53 more than 8-fold compared with LacZ (Supplementary Table S1). These genes included several known p53 targets such as CDKN1A, MDM2, LGALS7, PHLDA3, and so on. Among these genes, we focused on aldo-keto reductase family 1, member B10 (AKR1B10) as a candidate for novel p53 target genes. The expression of AKR1B10 was significantly increased following overexpression of p53, p63\(\gamma\), and p73\(\beta\) (Fig. 1A). To validate this induction of AKR1B10, we compared AKR1B10 mRNA expression in H1299 and SaOS-2 osteosarcoma cells (p53-null) overexpressing p53 family members and control cells by quantitative RT-PCR (Fig. 1B). The results indicated that the expression of AKR1B10 mRNA was significantly upregulated by p53, p63\(\gamma\), and p73\(\beta\) in both cell lines. Moreover, a large increase in AKR1B10 mRNA was observed in cells with forced expression with p53 as compared with p63\(\gamma\) or p73\(\beta\). Furthermore, we quantified the expression of AKR1B10 mRNA in isogenic colon cancer cells [HCT116 (p53\('+/+\)'), and HCT116 cells (p53\(−/−\)); Fig. 1C] and found that AKR1B10 mRNA was significantly upregulated following adriamycin treatment in HCT116 cells (p53\(++\)) as compared with HCT116 cells (p53\(−/−\)). Together, these results demonstrated that AKR1B10 is upregulated in a p53-dependent manner.

The binding of p53 family proteins to the AKR1B10 promoter and their transcriptional activity
To investigate whether the AKR1B10 gene is a direct target of transcriptional activation by p53 family members,
we analyzed the next-generation ChIP-seq data obtained in our previous study. We identified 2 ChIP-seq peaks in the upstream region of the AKR1B10 gene (Fig. 2A), and the sequence of each peak corresponded to a consensus p53 binding motif (RRRCWWGYYY RRRCWWGYYY) (RE1 and RE2 in Fig. 2A). Furthermore, we confirmed the interaction between p53 family member proteins and AKR1B10-RE1, RE2 by ChIP-PCR (Fig. 2B, top). We also detected the interaction between endogenous p53 and RE1, RE2 in HCT116 (p53+/+) cells (Fig. 2B, bottom). To determine whether the AKR1B10-RE1 and RE2 sequences confer transcriptional activity in a p53-dependent manner, we performed a reporter assay using luciferase vectors including AKR1B10-RE1 or RE2 sequences. SaOS-2 cells were transiently cotransfected with each luciferase vector together with a p53-, p63-, or p73B-expressing plasmid. For both reporters including RE1 and RE2 sequences, luciferase activity was increased in the presence of each of the tested p53 family members as compared with the control vector (MOCK; Fig. 2C, left). In addition, luciferase activities were significantly increased following adriamycin treatment in HCT116 (p53+/+) cells as compared with HCT116 (p53−/−) cells (Fig. 2C, right), demonstrating that the observed increase depended on wild-type p53. These results indicated that p53 family members directly transactivate the AKR1B10 gene through binding to p53 response elements.

The induction of AKR1B10 protein by p53 family members

Next, to assess whether AKR1B10 is induced by p53 family members at the protein level, DLD1 and SW480 colon cancer cell lines were infected with adenoviruses expressing p53 family members, and the expression of AKR1B10 protein was then examined by Western blotting (Fig. 3A). In both cell lines, the expression of AKR1B10 protein was increased in the presence of p53, p63γ, or p73B. Next, HCT116 (p53+/+, p53−/−), LoVo, and RKO (p53+/+) cells were treated with adriamycin, which activates endogenous p53 protein by inducing DNA damage

Figure 2. The response elements for p53 family members in the human AKR1B10 gene. (A) The genomic position of in silico p53 motifs and ChIP-seq peaks are indicated as gray bars and black paintings, respectively. The AKR1B10 gene is also displayed as boxes connected by horizontal lines; boxes indicate exons (wide box: open reading frame), horizontal lines indicate introns, and > marks on the line indicate the transcriptional direction. In silico p53 motif sequences corresponding to ChIP-seq peaks (RE1, RE2) are also displayed (bases matching and mismatching the consensus p53 motif [RRRCWWGYYY RRRCWWGYYY] are shown in upper and lower case letters, respectively). (B) H1299 cells were infected with adenoviral vectors expressing LacZ, FLAG-p53, FLAG-p63γ or FLAG-p73β at an MOI of 25. At 24 h after infection, ChIP was performed with an anti-FLAG antibody or IgG as a control (top). HCT116 (p53+/+) cells (Fig. 2B, bottom). (C) The relative luciferase activity of reporter vectors including RE1, RE2 (WT) or each mutant (Mut) transfected into SaOS-2 cells (p53 null) with plasmids expressing MOCK, p53, p63γ, or p73β (left). The relative luciferase activity of the same reporters is shown in HCT116 (p53+/+) or (p53−/−) cells with or without adriamycin treatment (0.5 μg/mL; right). The averages of 3 experiments are indicated. Error bars indicate the SD.
In HCT116 (p53+/+) and LoVo cells, adriamycin treatment increased the expression of AKR1B10 protein along with p53 in a dose-dependent manner, whereas the increase was weak in HCT116 (p53−−) cells. Although p73 protein was also activated by adriamycin, the increased level of p73 protein was considerably lower than that of p53. In addition, endogenous p63 protein was not detected in these cell lines (data not shown). These results indicated that endogenous p53 principally increased AKR1B10 protein. In RKO cells, AKR1B10 protein was induced more strongly following adriamycin treatment at a concentration of 0.5 μg/mL as compared with 1.0 μg/mL. This opposite result may have been caused by the high sensitivity of RKO cells to adriamycin-induced apoptosis, as PARP-1 cleavage, which is an indicator of apoptosis, was observed in RKO cells treated with 1.0 μg/mL of adriamycin.

The downregulation of AKR1B10 expression in human colorectal cancers

In contrast to the ubiquitous expression of aldose reductases, AKR1B10 is expressed mainly in the small intestine and colon (22). Therefore, to examine the change in AKR1B10 expression during colorectal carcinogenesis, we surveyed 4 gene expression datasets of human colorectal cancers and 2 datasets of colorectal adenomas in Oncomine, a cancer microarray database (www.oncomine.org/). The expression of AKR1B10 was decreased in colorectal cancers as compared with normal colon tissues, and this difference was statistically significant in all 4 datasets (Fig. 4A). Furthermore, AKR1B10 expression was also significantly decreased in colorectal adenomas, which are considered as precancerous lesions, as compared with normal colon tissues (Fig. 4B). Therefore, these results suggest that the downregulation of AKR1B10 contributes to colorectal carcinogenesis and cancer progression.

The knockdown of AKR1B10 inhibits p53-induced apoptosis

To evaluate the biological effect of AKR1B10 downregulation, we knocked down AKR1B10 by siRNA in BM314 cells infected with p53-expressing adenovirus as well as HCT116 cells treated with adriamycin (Fig. 5A). The level of p53-induced apoptosis was quantified by evaluating the sub-G1 population (Fig. 5B). Interestingly, knockdown of AKR1B10 significantly inhibited the growth of the sub-G1 population in both cell types. PARP-1 cleavage (Fig. 5A) and caspase-3 activity (Fig. 5C), which serve as other indicators of apoptosis, were also inhibited when AKR1B10 was knocked down in these cells. The activities of caspase-8 and -9 that were upstream of caspase-3 were also inhibited by AKR1B10 knockdown (Supplementary Fig. S1A). Furthermore, we confirmed that cotransfection of AKR1B10 siRNAs with AKR1B10 expression vector partially recovered the induction of apoptosis (Fig. 5D). AKR1B10 requires NADPH as the reducing cofactor (23). Although the knockdown of AKR1B10 slightly decreased NADP⁺/NADPH ratio, the decrease was not statistically significant (Supplementary Fig. S1B). To confirm whether these effects depend on the enzymatic activity of AKR1B10, we treated these cells with flufenamic acid (FFA), an enzymatic inhibitor of AKR1B10 (24) and quantified p53-induced apoptosis by flow cytometry (Fig. 5E). Consistent with the results for AKR1B10 knockdown, the level of p53-induced apoptosis was attenuated following treatment with flufenamic acid. These results indicate that AKR1B10 is involved in the p53-dependent pathway to induce apoptosis.
Figure 4. The expression levels of AKR1B10 in human colorectal cancers and adenomas. Four gene expression datasets of colorectal cancers (A) and 2 gene expression datasets of colorectal adenomas (B) were analyzed in Oncomine and displayed as a boxplot. (log2 median-centered). The sample numbers are shown in parentheses. P values were calculated using the Student t test.

The overexpression of AKR1B10 inhibits the proliferation of colon cancer cells

We established stable cell lines constitutively expressing AKR1B10 in LoVo and DLD1 cells by lentivirus-mediated gene transfer. The constitutive expression of AKR1B10 was confirmed by Western blotting in each cell line (Fig. 6A). To determine whether AKR1B10 overexpression would inhibit tumor growth in vivo, we examined tumor volume in a mouse xenograft model. LoVo and DLD-1 cells were injected into nude mice subcutaneously, and the volume of each tumor was subsequently measured (Fig. 6B). In both cell lines, the volume of tumors derived from AKR1B10-overexpressing cells was less than that of tumors derived from MOCK cells. This result indicated that AKR1B10 has an inhibitory effect on cell proliferation in colorectal cancers. Furthermore, the level of p53-induced apoptosis was quantified by evaluating the sub-G1 population in these cell lines (Fig. 6C). In contrast to the result obtained with AKR1B10 knockdown cells shown in Fig. 5, p53-induced apoptosis was significantly enhanced in AKR1B10-overexpressing cells compared with control cells.

The downregulation of AKR1B10 correlates with poor prognosis in colorectal cancer

To examine whether AKR1B10 expression affects prognosis in colorectal cancer, we surveyed 3 gene expression datasets of human colorectal cancers that included survival information, and survival curves were constructed using the Kaplan–Meier method (Fig. 7). In all datasets analyzed, the rate of disease-free or overall survival was significantly lower among patients whose tumors had low levels of AKR1B10 expression as compared with high levels. This result indicates that low expression of AKR1B10 may serve as a poor prognostic factor for patients with colorectal cancers.

Discussion

In this study, we identified AKR1B10 as a direct transcriptional target of p53, as AKR1B10 mRNA was significantly induced by p53 (Fig. 1). The promoter region of the AKR1B10 gene contains 2 p53REs, both of which interacted with p53 family proteins (Fig. 2A and B). However, the number of p53 motif sequences corresponding to ChIP-seq peaks in RE1 and RE2 (consensus p53 motif: RRCWWGGYYY) was only 15 and 14, respectively (Fig. 2A). In addition, the increase in
Figure 5. The effect of AKR1B10 knockdown on p53-induced apoptosis. A, BM314 and HCT116 cells were transfected with siRNAs targeting AKR1B10 (si-AKR1B10+/−) or the negative control (si-AKR1B10/C0). At 4 hours after transfection, BM314 cells were infected with adenoviral vectors expressing p53 (p53+/−) or LacZ (p53−) as a control at an MOI of 200, and HCT116 cells were treated with or without adriamycin (0.5 μg/mL). At 24 hours after viral infection or drug treatment, siRNAs were again transfected into these cells. At 24 hours after the second transfection, total cell lysates were analyzed by Western blotting with the indicated antibodies. Under the same conditions used in (A), cells were analyzed by flow cytometry. The percentage of cells in the sub-G1 phase is indicated (B). Caspase-3 activity was also measured using the Caspase-3 Colorimetric Assay Kit (C). The averages of 3 independent experiments are indicated. (D) BM314 cells were transfected with siRNAs targeting AKR1B10 (si-AKR1B10+/−) or the negative control (si-AKR1B10/C0), and plasmid expressing AKR1B10 (pcDNA3.2-AKR1B10, AKR1B10+/+) or its MOCK (AKR1B10/C0). At 4 hours after transfection, cells were infected with adenoviral vectors expressing p53. At 48 hours after infection, cells were analyzed by flow cytometry. The percentage of cells in the sub-G1 phase is indicated. Total cell lysates were also analyzed by Western blotting with the indicated antibodies. E, BM314 cells were infected with adenoviral vectors expressing p53 or LacZ as a control at an MOI of 200. At 4 hours after infection, cells were treated with the AKR1B10 inhibitor FFA [0 (−) or 200 μmol/L (+)]. At 48 hours after treatment, cells were analyzed by flow cytometry. The percentage increase in sub-G1 phase cells by p53 (p53−/LacZ) is indicated. The averages of 3 independent experiments are indicated. In B–E, error bars indicate the SE. * indicates a P value < 0.05 by t test. F, under the same conditions used in (A), mRNA expression in HCT116 cells was analyzed by microarray. Genes with greater than 2-fold increased expression in cells treated with adriamycin as compared with untreated cells were selected, and hierarchical clustering analysis was performed. The red and green colors in the heat map indicate positive and negative Z-scores, respectively. * indicates the gene cluster that was increased only in control cells and not in AKR1B10-knockdown cells.
transcriptional activity observed in the reporter assay was not as high for plasmid vectors including only the RE1 or RE2 sequence (Fig. 2C), which suggests that a synergistic response between these REs for p53 family proteins may be responsible for the high level of transactivation observed.

AKR1B10 is a member of the aldo-keto reductase (AKR) superfamily, which is composed of several enzymes that catalyze redox transformations involved in biosynthesis, intermediary metabolism, and detoxification. One characteristic feature of AKRs is their ability to catalyze aldehyde or ketone

Figure 6. The effect of AKR1B10 overexpression on the proliferation of colorectal cancers. A, LoVo and DLD1 cells were stably transfected with lentiviral vectors expressing AKR1B10 or LacZ as a control. The constitutive expression of AKR1B10 was confirmed by Western blotting. B, the indicated AKR1B10-overexpressing or control cells were injected subcutaneously into nude mice. The volume of each tumor was quantified, and the average volume of 3 independent tumors was plotted. C, the indicated AKR1B10-overexpressing or control cells were infected with adenoviral vectors expressing p53 (p53+) or LacZ (p53−) as a control at an MOI of 100. At 48 hours after infection, cells were analyzed by flow cytometry. The percentage of cells in the sub-G0 phase is indicated. In B and C, error bars indicate the SE. * indicates a P value < 0.05 by t test.

Figure 7. The correlation between AKR1B10 expression and prognosis among colorectal cancer patients. For the 3 indicated datasets, the correlation between AKR1B10 expression and survival was analyzed and plotted using the Kaplan–Meier method. The survival rates for patients with high and low AKR1B10 expression are plotted as solid and dashed lines, respectively. The number of patients in each group is shown in parentheses. P values were calculated by log-rank test.
reduction (23). In addition, the amino acid sequence of AKR1B10 is 71% identical to that of aldose reductase, and this enzyme exhibits substrate specificity and inhibitor sensitivity similar to aldose reductase (22). In contrast to ubiquitously expressed AKRs, AKR1B10 is mainly expressed in normal small intestine and colon, which suggests that the physiological function of AKR1B10 is specifically required in intestinal tissues (22). The expression of AKR1B10 was significantly decreased in colorectal cancer tissue as compared with the normal colon (Fig. 4), and mutation in p53 was likely the primary reason for this decrease because AKR1B10 is a direct transcriptional target of p53. However, the rate of p53 mutation in colorectal cancers is approximately 40% (2), whereas the expression of AKR1B10 is decreased in most colorectal cancers (Fig. 4A). In addition, decreased AKR1B10 expression was also observed in colorectal adenomas, whose rate of p53 mutation was lower than that of colorectal carcinomas (Fig. 4B). These results suggest that the decrease in AKR1B10 expression does not necessarily depend on p53 mutation.

It is well known that DNA methylation of CpG islands in promoter regions causes the inactivation of gene expression in various carcinomas (25). However, the promoter region of the AKR1B10 gene does not contain a CpG island. Furthermore, the public database of genome-wide DNA methylation according to reduced representation bisulfate sequencing revealed no significant difference in the status of DNA methylation in the promoter regions of the AKR1B10 gene between colorectal cancers and normal colon tissue (26). Therefore, decreased AKR1B10 expression in colon cancer may be caused by other epigenetic alterations, such as histone modification in the promoter region, or by changes in other transcriptional factors that are required to specifically transactivate the AKR1B10 gene in the normal colon and small intestine.

AKR1B10 is expressed at very low levels in normal lung, breast, and pancreatic tissues (27), whereas ectopic overexpression of AKR1B10 has been reported in lung small cell cancers, breast cancers, pancreatic cancers, liver cancers, and cervical cancers (28–32). These inconsistent results indicate that AKR1B10 likely has different roles in different types of cancers; in particular, we speculate that AKR1B10 has an inhibitory effect on colorectal carcinogenesis and progression, whereas its function may be favorable for other cancers. Because AKR1B10 is a metabolic enzyme that catalyzes ald-o-keto reduction, it may depend on the tissue type or the intracellular metabolic status as to whether changes in catalytic activity have a positive or negative effect on cancer growth. Recently, the relationship between p53 and metabolism has received a great deal of attention (5, 33–35), and the role of AKR1B10 as a target of p53 should be taken into consideration for the analysis of the p53-related metabolome.

We discovered that the downregulation of AKR1B10 inhibited p53-induced apoptosis (Fig. 5A–C), whereas the overexpression of AKR1B10 enhanced p53-induced apoptosis (Fig. 6C) in colon cancer cells. Furthermore, treatment with flufenamic acid, an enzymatic inhibitor of AKR1B10, also suppressed p53-induced apoptosis (Fig. 5E), which indicates that p53-induced apoptosis is partially affected by the enzymatic activity of AKR1B10. In addition, we detected a gene cluster that was increased after DNA damage in control cells, but not AKR1B10-knockdown cells, following treatment with a DNA-damaging agent (Fig. 5F). However, AKR1B10 was not found to affect p53-induced transactivation directly, as 128 genes in this cluster did not include any known p53 target genes (Supplementary Table S2). Although the precise mechanism remains unclear, it can be speculated that changes in metabolic status catalyzed by AKR1B10 modulate the pattern of gene induction by DNA damage (Fig. 5F), which could lead to changes in the apoptotic response. We also found that the expression of AKR1B10 was decreased in most colorectal cancers (Fig. 4A), whereas the normal function of p53 is preserved in approximately one-half of colorectal cancers. Therefore, we believe that the inhibition of p53-induced apoptosis, resulting from the suppression of AKR1B10, contributes to the decreased survival rate of patients with colon cancer (Fig. 7).

We also found that the overexpression of AKR1B10 suppressed the proliferation of colorectal cancer cells in vivo (Fig. 6B), and this suppression was not because of the inhibition of p53-induced apoptosis because this result was independent of p53 mutation status. Instead, the change in metabolic status catalyzed by AKR1B10 may directly induce this proliferative suppression. Therefore, the biological consequences of AKR1B10 suppression, that is, the inhibition of p53-induced apoptosis and proliferative suppression, likely contribute to colorectal carcinogenesis and progression.

In this study, we demonstrated that decreased AKR1B10 expression induced the inhibition of p53-induced apoptosis and proliferative suppression, which led to decreased survival rates in colorectal cancer patients. These results suggest that AKR1B10 expression may be useful for prognostic prediction and that interventions focused on metabolic pathways involving AKR1B10 may represent novel therapeutic approaches for colorectal cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: T. Ohashi, M. Idogawa, T. Tokino
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T. Ohashi, M. Idogawa
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T. Ohashi, M. Idogawa
Writing, review, and/or revision of the manuscript: T. Ohashi, M. Idogawa, T. Tokino
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Idogawa, Y. Sasaki, H. Suzuki
Study supervision: M. Idogawa

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

Received June 19, 2013; revised September 4, 2013; accepted October 2, 2013; published OnlineFirst October 18, 2013.
References


AKR1B10, a Transcriptional Target of p53, Is Downregulated in Colorectal Cancers Associated with Poor Prognosis

Tomoko Ohashi, Masashi Idogawa, Yasushi Sasaki, et al.


Updated version Access the most recent version of this article at: doi:10.1158/1541-7786.MCR-13-0330-T

Supplementary Material Access the most recent supplemental material at: http://mcr.aacrjournals.org/content/suppl/2013/10/18/1541-7786.MCR-13-0330-T.DC1

Cited articles This article cites 34 articles, 6 of which you can access for free at: http://mcr.aacrjournals.org/content/11/12/1554.full.html#ref-list-1

Citing articles This article has been cited by 3 HighWire-hosted articles. Access the articles at: /content/11/12/1554.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.