Foxo3a Regulates Apoptosis by Negatively Targeting miR-21*

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MicroRNAs are a class of small non-coding RNAs that mediate post-transcriptional gene silencing. Recently, the work on miRNAs renovates our understanding about apoptotic regulation. They can be classified as either pro- or anti-apoptotic miRNAs (1, 2). For example, miR-1 participates in the initiation of apoptosis (3), whereas miR-21 is able to inhibit apoptosis (4). miRNAs are expressed at a constant level under physiological condition, and their functions depend on their expression levels. It remains a challenging question as to how their expression is regulated in the apoptotic program.

The forkhead family of transcription factors is characterized by the presence of a conserved 100-amino acid DNA binding domain and participate in regulating diverse cellular functions such as apoptosis, differentiation, metabolism, proliferation, and survival (5). Foxo3a is a substrate of protein kinase Akt, and its transcriptional output is controlled via phosphorylation. In the absence of cellular stimulation and when Akt is inactive, Foxo3a is localized within the nucleus where it performs transcription of target genes. However, upon phosphorylation by Akt at Thr-32, Ser-253, and Ser-315, it binds to 14-3-3 and cannot exert the transcriptional function (6).

Fas ligand (FasL) is a potential transcriptional target of Foxo3a, but the transcriptional output can be either activation or suppression. It has been reported that Foxo3a can stimulate FasL expression, thereby triggering apoptosis (6). However, there is also evidence showing that Foxo3a decreases the expression of FasL (7). The molecular mechanism by which Foxo3a regulates FasL expression remains further elusive.

miR-21 has been shown to regulate apoptosis by targeting a variety of apoptotic factors. It contributes to glioma malignancy by down-regulating matrix metalloproteinase inhibitors, thereby leading to the activation of matrix metalloproteinases and promoting invasiveness of cancer cells (8). It promotes cell transformation by targeting the programmed cell death 4 gene (9). miR-21 is overexpressed in human cholangiocarcinoma and regulates programmed cell death 4 and the tissue inhibitor of metalloproteinase 3 (10). Given the important role of FasL in apoptosis, it is not yet clear whether FasL can be a target of miR-21 in the apoptotic machinery.

The expression levels of miRNAs remain constant under physiological condition. Their alterations may cause the patho- logical disorders. miRNA expression can be regulated by transcriptional factors. For example, miR-34a causes dramatic reprogramming of gene expression and promotes apoptosis, and it is transactivated by the tumor suppression gene p53 (11). Serum response factor can directly bind to the promoter of miR-1-1 and miR-1-2 genes and activate their expression (12). Our recent work reveals that miR-23a is a transcriptional target of nuclear factor of activated T cells c3 (13). Because the functions of miRNAs are closely related to their expression levels, it is important to explore the molecular mechanism governing their expression. Foxo3a and miR-21 are involved in apoptosis, but it is not yet clear whether there is an impact between these two factors in the apoptotic machinery.

Lung cancer is the most common cause of cancer-related death. Chemotherapy plays an important role for the treatment of lung cancer, and doxorubicin is commonly used for lung cancer therapy. However, a major obstacle of chemotherapy is the cancer resistance. It is critically important to identify the factors that participate in the regulation of cancer cell apoptosis, so that novel approaches can be developed for cancer therapy.

Our present work aimed at elucidating the molecular mechanism by which Foxo3a regulates the apoptotic program in A549 human lung cancer cells. Our results revealed that Foxo3a
transcriptionally represses the expression of miR-21. Furthermore, we identified Fasl as a target of miR-21. In addition, we found that Foxo3a regulates Fasl through miR-21. Our data shed new light on understanding the regulation of miRNA expression and the relationship among Foxo3a, miR-21, and Fasl in the apoptotic cascades.

EXPERIMENTAL PROCEDURES

Cell Cultures and Treatment—Human lung cancer cells (A549) and human neuroblastoma cells (SH-EP1) were grown in Dulbecco’s modified Eagle’s medium; Invitrogen) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin in a humidified atmosphere of 5% CO₂ at 37 °C. The treatment with doxorubicin (Sigma) was performed as described (14). The culture of cardiac fibroblasts was performed as described (15).

Cell Viability and Apoptosis Assays—Cell death was determined by trypan blue exclusion, and the numbers of trypan blue-positive and -negative cells were counted on a hemocytometer. Apoptosis was determined by the terminal deoxynucleotidyltransferase-mediated TUNEL using a kit from Roche Applied Science. The detection procedures were in accordance with the kit instructions.

Constructions of Foxo3a and Fasl RNAi Interference (RNAi)—The Foxo3a RNAi sense sequence is 5’-GAGCTCTTGGTGATGATCCACCAAGAGCTC-3’, the antisense sequence is 5’-GATGATCCACCAAGAGCCTACCATACCTCA-3’. The scramble Foxo3a RNAi sense sequence is 5’-GGCGTATGGAGATCATTA-3’, the scramble antisense sequence is 5’-TGAGAAGCTACATACGACACCC-3’. Fasl RNAi sense sequence is 5’-AAGGAGCTGAGGAAAGTG-3’, the antisense sequence is 5’-CAGCTTCTCAGCTCTTCTT-3’. The scramble Fasl RNAi sense sequence is 5’-TGGAGAGCAGTAGAGAGAG-3’, the scramble antisense sequence is 5’-CTCTCTACTGTCTCTTCCA-3’. They were cloned into pSilencer adeno 1.0-CMV vector (Ambion) according to the manufacturer’s instructions.

Constructions of miR-21 and Fasl Promoters—Human mir-21 promoter region was amplified from human genomic DNA using PCR to generate wild type promoter. The large fragment containing two Foxo3a potential binding sites (wild type promoter-1, wt-1) was amplified using the forward primer, 5’-AAACCAAGGCTCTTACCATAGC-3’. The scramble Foxo3a potential binding site in wt-1 fragment was amplified using the reverse primer, 5’-ATAAGCTA-3’. The short fragment containing one Foxo3a potential binding site (wild type promoter-2 (wt-2)) was amplified using the forward primer, 5’-GCTGATGAGAGCAGTACCTACCAAG-3’. Both fragments were amplified using the reverse primer, 5’-TGGTACACCCATGGAGATGTCAC-3’. The promoters were cloned into the reporter plasmid, pGL4.17 (Promega). The introduction of mutations in the putative Foxo3a binding site in wt-1 fragment (−197 to −191 wild type, 5’-ATATATA-3’; mutant, 5’-ACGGCCA-3’) was generated using QuikChange II XL site-directed mutagenesis kit (Stratagene). Human Fasl promoter region (from −3157 to +2) was amplified using the same protocol as described for miR-21. The forward primer was 5’-GTGACCTGTCCACATTACACAGC-3’, the reverse primer was 5’-CTGATGAGCCAGCTGGTAGTCAG-3’. The constructs were sequence-verified.

Preparation of miR-21 Expression Construct—miR-21 was synthesized by PCR using human genomic DNA as the template. The upstream primer was 5’-GCATTATGAGCATTATGTCAGA-3’; the downstream primer was 5’-CATACACGCTAGAAAAGTCCCCGTG-3’. The PCR fragment was finally cloned into the Adeno-X™ Expression System (Clontech) according to the manufacturer’s instructions.

Preparation of the Luciferase Construct of Fasl 3’-UTR—Fasl with 3’-UTR was amplified by PCR. The forward primer was 5’-GGAGACCCATTTGAGATGTCATATTC-3’, the reverse primer was 5’-CCCTAATTGCCACCTGGAAATAC-3’. The PCR fragment was subcloned into the pGL3 vector (Promega) immediately downstream of the stop codon of the luciferase gene. To produce mutated 3’-UTR, the mutations (wild type 3’-UTR, 5’-ATAAGCTA-3’; mutated 3’-UTR: 5’-ATCCATTA-3’) were generated using QuikChange II XL site-directed mutagenesis kit (Stratagene). The constructs were sequence-verified. They were cloned into the Adeno-X™ Expression System (Clontech) according to the manufacturer’s instructions.

Immunoblot and Immunofluorescence—Immunoblotting was carried out as we previously described (16). Cells were lysed for 1 h at 4 °C in a lysis buffer (20 mM Tris, pH 7.5, 2 mM EDTA, 3 mM EGTA, 2 mM dithiothreitol, 250 mM sucrose, 0.1 mM phenylmethylsulfonyl fluoride, 1% Triton X-100) containing a protease inhibitor mixture. Samples were subjected to SDS-PAGE and transferred to nitrocellulose membranes. Equal protein loading was controlled by Ponceau Red staining of membranes. Blots were probed using the primary antibodies. The anti-Foxo3α and the anti-phospho Foxo3α antibody (Thr-32) were from Cell Signaling. The anti-Fasl antibody was from Abcam. The anti-PCNA (proliferating cell nuclear antigen) antibody and the anti-actin antibody were from Santa Cruz Biotechnology. After four washes with phosphate-buffered saline, Tween 20, the horseradish peroxidase-conjugated secondary antibodies were added. Antigen-antibody complexes were visualized by enhanced chemiluminescence. Immunofluorescence was performed as we described (17). The samples were imaged using a laser scanning confocal microscope (Zeiss LSM 510 META).

Preparation of Subcellular Fractions—Subcellular fractions were prepared as described with modifications (18). In brief, cells were washed twice with phosphate-buffered saline, and the pellets were suspended in 0.2 ml of ice-cold buffer (20 mM potassium-HEPES, pH 7.8, 5 mM potassium acetate, 0.5 mM MgCl₂, and 0.5 mM dithiothreitol) containing a protease inhibitor mixture. The cells were homogenized by 12 strokes in a Dounce homogenizer. The homogenates were centrifuged at 4 °C for 5 min at 4 °C to collect nuclei. The resulting supernatants were centrifuged at 20,000 × g for 5 min at 4 °C to collect the cytosolic fractions.

Chromatin Immunoprecipitation (ChIP) Analysis—ChIP was performed as we and other described (19, 20). In brief, cells were washed with phosphate-buffered saline and incubated for
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10 min with 1% formaldehyde at room temperature. The crosslinking was quenched with 0.1 M glycine for 5 min. Cells were washed twice with phosphate-buffered saline and lysed for 1 h at 4 °C in a lysis buffer. The cell lysates were sonicated into chromatin fragments with an average length of 500–800 bp as assessed by agarose gel electrophoresis. The samples were pre-cleared with Protein A-agarose (Roche Applied Science) for 1 h at 4 °C on a rocking platform, and 5 μg of specific antibodies were added and rocked for overnight at 4 °C. The anti-NFAT4 antibody was from Santa Cruz Biotechnology. Immunoprecipitates were captured with 10% (v/v) Protein A-agarose for 4 h. Before use, Protein A-agarose was blocked twice at 4 °C with 5% skim milk for 1 h. Cells were then washed three times with IP buffer (150 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 7.5) and once with 1% formaldehyde. The cross-linking was quenched with 0.1 M glycine for 5 min. Cells were then washed three times with cold PBS. The cells were harvested and lysed in lysis buffer (150 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 7.5, and 0.1% SDS). The nuclear proteins were then isolated. The proteins were separated on 7.5% SDS-PAGE gels and transferred to a nitrocellulose membrane. The membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) and incubated with primary antibodies (NFAT4, 1:1000, Santa Cruz Biotechnology; Phospho-NFAT4, 1:1000, Cell Signaling Technology) overnight at 4 °C. After washing, the membranes were incubated with the corresponding HRP-conjugated secondary antibodies (1:5000, Santa Cruz Biotechnology) for 1 h at room temperature. The blots were then developed using the ECL system (Millipore). The proteins were analyzed with the ImageQuant software (GE Healthcare). The experiments were repeated at least three times, and the results were expressed as means ± S.E. of at least three independent experiments. The statistical comparison among different groups was performed by one-way analysis of variance (ANOVA). Paired data were evaluated by Student’s t test. p < 0.05 was considered statistically significant.

RESULTS

Foxo3a Relocalization to the Nucleus and miR-21 Down-regulation Simultaneously Occur upon Doxorubicin Treatment—We analyzed whether Foxo3a and miR-21 are involved in the apoptotic program of doxorubicin. The immunofluorescence of A549 cells revealed that Foxo3a was predominantly distributed in the cytoplasm in the control cells without treatment. In contrast, Foxo3a was accumulated in the nuclei in response to doxorubicin treatment. The immunoblotting of subcellular fractions also revealed that Foxo3a was translocated from the cytoplasm to the nuclei upon doxorubicin treatment (Fig. 1A). Because doxorubicin is a component of the standard chemotherapeutic protocol to treat neuroblastoma, we tested whether it can also influence Foxo3a in the human neuroblastoma cell line SH-EP1. A similar result was obtained in SH-EP1 cells (Fig. 1B). The subcellular distributions of Foxo3a are controlled by its phosphorylation status (6). Accordingly, we detected the subcellular distributions of the phosphorylated and nonphosphorylated Foxo3a upon doxorubicin treatment. We observed a time-dependent elevation of nonphosphorylated Foxo3a in the nuclei and a reduction of phosphorylated Foxo3a in the cytoplasm in both A549 and SH-EP1 cells (Fig. 1C). These data suggest that doxorubicin can induce the relocalization of Foxo3a to the nuclei.

We detected the levels of miR-21 in cells upon doxorubicin treatment. Doxorubicin induced a reduction in miR-21 levels in A549 (Fig. 1D) and SH-EP1 cells (Fig. 1E). To know if the alterations of miR-21 is specific or not, we detected miR-670, and no significant alterations in miR-670 levels were observed (data not shown). Thus, it appears that miR-21 can be down-regulated by doxorubicin.

Foxo3a Can Negatively Regulate miR-21 Expression—The simultaneous alterations in Foxo3a and miR-21 led us to consider whether these two events are related. To address this issue, we produced Foxo3a RNAi constructs and tested whether knockdown of Foxo3a can influence miR-21 levels.
The RNAi construct of Foxo3a but not its scramble form was able to suppress Foxo3a expression (Fig. 2A). Surprisingly, knockdown of Foxo3a could attenuate the reduction of miR-21 induced by doxorubicin in A549 (Fig. 2B) and SH-EP1 cells (Fig. 2C). To further understand whether Foxo3a is able to influence miR-21 expression, we detected miR-21 levels in cells expressing the constitutively active form of Foxo3a (caFoxo3a) (Fig. 2D). Enforced expression of caFoxo3a led to an elevation in miR-21 levels in A549 (Fig. 2E) and SH-EP1 cells (Fig. 2F). These data suggest that Foxo3a can negatively influence the expression of miR-21.

Foxo3a and miR-21 Cross-talk in the Apoptotic Program—We tested whether Foxo3a and miR-21 are functionally related in apoptosis. Enforced expression of miR-21 could attenuate apoptosis induced by caFoxo3a in A549 (Fig. 3A) and SH-EP1 cells (Fig. 3B). miR-21 also could inhibit apoptosis induced by...
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FIGURE 3. Foxo3a and miR-21 cross-talk in apoptosis. A and B, enforced expression of miR-21 attenuates apoptosis induced by caFoxo3a. A549 (A) or SH-EP1 cells (B) were co-infected with the adenoviral construct of caFoxo3a or miR-21. The adenovirus containing β-galactosidase (β-gal) was used as a control. Apoptosis was analyzed by TUNEL assay 48 h after infection. TUNEL-positive cells were counted under the fluorescent microscope according to the kit instructions. *, p < 0.05 versus caFoxo3a alone. C, enforced expression of miR-21 inhibits apoptosis induced by doxorubicin. A549 cells were infected with the adenoviral construct of miR-21 or β-gal. Cells were treated with 2 μM doxorubicin 24 h after infection. A TUNEL assay was performed 12 h after doxorubicin in treatment. The percentages of TUNEL-positive cells are shown in the left panel. *, p < 0.05 versus doxorubicin alone. Representative photos of TUNEL staining are shown in the right panel. Bar = 50 μm. The nuclei with the green color are TUNEL-positive. The nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, blue). D, knockdown of miR-21 sensitizes doxorubicin to inducing apoptosis is shown. A549 cells were transfected with miR-21 antagonist or the antagonist-NC. 24 h after transfection were treated with 0.2 μM doxorubicin. A TUNEL assay was performed 12 h after doxorubicin treatment. *, p < 0.05 versus doxorubicin alone or antagonist alone. E, knockdown of endogenous Foxo3a attenuates apoptosis induced by doxorubicin is shown. A549 cells were infected with the RNAi constructs of Foxo3a or its scramble form (Foxo3a-S-RNAi). Cells were then transfected with miR-21 antagonist or the antagonist-NC. 24 h after transfection were treated with 2 μM doxorubicin. Apoptosis was analyzed by TUNEL assay. *, p < 0.05 versus doxorubicin alone. Data are expressed as the mean ± S.E. of three independent experiments.

doxorubicin (Fig. 3C). We characterized the role of endogenous miR-21 in the apoptotic program of doxorubicin. Doxorubicin at a dose of 0.2 μM led to a less amount of cells undergoing apoptosis. However, knockdown of miR-21 resulted in a significant amount of cells undergoing apoptosis in response to the same dose of doxorubicin treatment (Fig. 3D). We tested whether Foxo3a is involved in mediating the apoptotic signal of doxorubicin. Knockdown of Foxo3a was able to attenuate apoptosis induced by doxorubicin, but this effect could be abolished by miR-21 antagonir (Fig. 3E). Collectively, Foxo3a and miR-21 are functionally related in the apoptotic cascades. miR-21 Is a Transcriptional Target of Foxo3a—The functional correlation between Foxo3a and miR-21 necessitates the elucidation of the mechanism by which miR-21 is regulated by Foxo3a. Foxo3a is a transcription factor. Accordingly, we tested whether the regulation of miR-21 by Foxo3a occurs through a transcriptionally dependent or independent manner. To this end, we analyzed the promoter region of miR-21. There are two optimal Foxo3a consensus binding sites (23). We sequenced the promoter region of FasL (3157 bp) and tested whether NFAT4 can bind to the promoter region of miR-21. The ChIP assay revealed an increase in the association levels of Foxo3a with BS2 in response to doxorubicin treatment. However, an association of Foxo3a with BS1 was not detectable (Fig. 4A). We tested whether Foxo3a can influence miR-21 promoter activity. Wild type miR-21 promoter (wt-1) demonstrated a low activity in the presence of caFoxo3a. Also, the truncated form of wild type miR-21 promoter containing only the binding site-2 (wt-2) showed a low activity in the presence of caFoxo3a. However, mutations in the Foxo3a consensus binding site-2 (BS2) could abolish the inhibitory effect of Foxo3a on miR-21 promoter activity (Fig. 4C). These data suggest that BS2 is the Foxo3a binding site.

Doxorubicin could induce a time-dependent reduction in miR-21 promoter activity (Fig. 4D). Concomitantly, knockdown of Foxo3a could attenuate the reduction of miR-21 promoter activity induced by doxorubicin in A549 (Fig. 4E) and SH-EP-1 cells (Fig. 4F). These data indicate that miR-21 can be transcriptionally repressed by Foxo3a. The Regulation of FasL by Foxo3a Is Not in a Transcription Manner—Foxo3a and miR-21 cannot directly execute apoptosis. Which molecules are their downstream mediators? Foxo3a has been shown to either activate or suppress FasL transcription depending on the cellular context (6, 7). We detected the levels of FasL in response to doxorubicin treatment. An up-regulation of FasL could be observed upon doxorubicin treatment (Fig. 5A). Knockdown of FasL attenuated cell death (Fig. 5B), suggesting that FasL participates in conveying the death signal of doxorubicin.

We tested whether the up-regulation of FasL is related to Foxo3a. Knockdown of Foxo3a attenuated FasL protein levels upon doxorubicin treatment (Fig. 5C). Surprisingly, FasL mRNA levels were not significantly altered by knockdown of Foxo3a (Fig. 5D). Human FasL promoter region contains 2 Foxo3a consensus binding sites (Fig. 5E). We performed a ChIP assay to detect whether Foxo3a binds to these sites upon doxorubicin treatment. The ChIP assay revealed that there was no detectable association between Foxo3a and the consensus binding site-1 (BS1) or binding site-2 (BS2) (Fig. 5F). We tested whether caFoxo3a can influence FasL mRNA levels and promoter activity. caFoxo3a led to no significant alterations in FasL mRNA levels (Fig. 5G) as well as FasL promoter activity (Fig. 5H). We sequenced the promoter region of FasL (3157 bp) and found no mutations in this region. We used NFAT4 as a positive control because FasL promoter contains NFAT4 binding sites (23). We tested whether NFAT4 can bind to the promoter region of FasL in the primary cardiac fibroblasts. NFAT4 asso-
FasL expression. miR-21 attains its effect in the presence of miR-21, as the expression levels of Foxo3a were detected by immunoblotting (Fig. 6E). These data suggest that miR-21 can inhibit the translation of FasL.

**DISCUSSION**

The growing evidence has shown that miR-21 is an oncogenic miRNA and is highly expressed in a variety of malignant tumors (24, 25). It is still a challenging question as to how the expression of miRNAs is regulated. Our present work demonstrated that miR-21 can be transcriptionally suppressed by Foxo3a. We further identified FasL as a target of miR-21. In particular, we found that Foxo3a can up-regulate FasL through down-regulating miR-21.
Our results provide novel evidence revealing that miR-21 can be a linker between Foxo3a and Fasl, and Foxo3a may promote apoptosis by targeting the anti-apoptotic miRNA, miR-21.
high activity of Akt. Future studies are required to test this hypothesis.

It is of note that miR-21 contributes to cancer resistance to therapies. For example, miR-21 is overexpressed in glioblastoma and mediates chemoresistance to the chemotherapeutic agent VM-26 in glioblastoma cells (32). The tumor necrosis factor-related apoptosis-inducing ligand in combination with miR-21 suppression leads to a synergistic increase in caspase activity and a decrease in cell viability in human glioma cells (33). Our data show that Foxo3a can transcriptionally down-regulate miR-21 and warrant future studies to develop a therapeutic approach to target miR-21 by modulating Foxo3a in cancer treatment.

Our present work revealed that the transcriptional factor Foxo3a can suppress miR-21 expression. There are reports showing that miR-21 can be transcriptionally up-regulated. The transcription factor AP-1 plays a key role in tumorigenesis, and it can induce miR-21 expression (34). Signal transducer and activator of transcription 3 (Stat3) is implicated in the pathogenesis of many malignancies and essential for interleukin-6-dependent survival and growth of multiple myeloma cells. Interleukin-6 can induce miR-21 expression through Stat3 (4). It is possible that miR-21 can also be directly or indirectly regulated by other transcriptional factors in the apoptotic cascades. For example, p53 activation leads to Foxo3a phosphorylation and subcellular localization changes that result in inhibition of Foxo3a transcription activity (35). It would be interesting to elucidate whether p53 can target miR-21 through Foxo3a.

Foxo3a is able to transcriptionally repress gene expression (7). Our present work revealed that Foxo3a could transcriptionally repress miR-21. Other transcriptional factors also can either activate and/or suppress gene expression. For example, in response to p53 transcriptional stimulation, 38 genes are up-regulated, and 24 genes are down-regulated (36). p53 can up-regulate the pro-apoptotic factors such as Bax (37), PUMA (38, 39), and Bad (40) but down-regulate the anti-apoptotic factors such as glutathione S-transferase-α (41) and Survivin (42).

Our present study demonstrated that Fasl is a target of miR-21. miR-21 has been shown to suppress other apoptotic proteins. For example, it can suppress tissue inhibitor of metalloproteinases 3 (10). miR-21 targets the network of p53, transforming growth factor-β, and mitochondrial apoptosis tumor suppressor genes in glioblastoma cells (43). miR-21 promotes glioma invasion by targeting matrix metalloproteinase regulators (8). Programmed cell death 4 is an important functional target of miR-21 in breast cancer (44) and colorectal cancer (45). Apoptosis can be initiated through the extrinsic and intrinsic pathways. In particular, there is cross-talk between these two pathways (46–48). Fasl can trigger the activation of extrinsic apoptotic pathway by promoting DISC formation (49). It is necessary to elucidate the consequences of miR-21 regulation on Fasl in apoptotic cascades.

Doxorubicin is an important component for the treatment of lung cancer and neuroblastoma. Induction of cancer cells to undergo apoptosis is a cellular basis of doxorubicin effect. However, a major obstacle of doxorubicin treatment is cancer resistance. Our present work demonstrated that miR-21 is negatively regulated by Foxo3a in both A549 and SH-EP1 cells upon doxorubicin treatment. We identified Fasl as a new target of miR-21. Furthermore, Foxo3a can regulate Fasl through miR-21. Future studies are required to delineate whether doxorubicin has a similar effect in other types of cancer cells. Overall, based on our findings, combinatorial therapies for cancer treatment may be developed by targeting the Foxo3a-miR-21-Fasl pathway.
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