LETTERS

Modulation of microRNA processing by p53

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MicroRNAs (miRNAs) have emerged as key post-transcriptional regulators of gene expression, involved in diverse physiological and pathological processes. Although miRNAs can function as both tumour suppressors and oncogenes in tumour development[1], a widespread downregulation of miRNAs is commonly observed in human cancers and promotes cellular transformation and tumorigenesis[2–4]. This indicates an inherent significance of small RNAs in tumour suppression. However, the connection between tumour suppressor networks and miRNA biogenesis machineries has not been investigated in depth. Here we show that a central tumour suppressor, p53, enhances the post-transcriptional maturation of several miRNAs with growth-suppressive function, including miR-16-1, miR-143 and miR-145, in response to DNA damage. In HCT116 cells and human diploid fibroblasts, p53 interacts with the Drosha processing complex through the association with DEAD-box RNA helicase p68 (also known as DDX5) and facilitates the processing of primary miRNAs to precursor miRNAs. We also found that transcriptionally inactive p53 mutants interfere with a functional assembly between Drosha complex and p68, leading to attenuation of miRNA processing activity. These findings suggest that transcription-independent modulation of miRNA biogenesis is intrinsically embedded in a tumour suppressive program governed by p53. Our study reveals a previously unrecognized function of p53 in miRNA processing, which may underlie key aspects of cancer biology.

In mammalian miRNA biogenesis, the primary transcripts of miRNA genes (pri-miRNAs) are cleaved into hairpin intermediates (pre-miRNAs) by the nuclear RNase III Drosha and further processed to mature miRNAs by cytosolic Dicer, another RNase-III-related enzyme[5]. The Drosha complex comprises Drosha, the DiGeorge syndrome critical region gene 8 (DGCR8) and multiple RNA-associated proteins including the DEAD box RNA helicases p68 and p72 (the latter also known as DDX17)[6]. p68 and p72 are required for the maturation of some, but not all, miRNAs[7].

A widespread decrease in mature miRNAs is often observed in various human malignancies[2,8–10]. In addition to genomic and epigenetic alterations[9,11], the deregulation might be attributable to the impairment of miRNA-processing steps[12,13]. Although several reports have described altered expression of miRNA-processing factors in tumour tissues[14], these phenomena may not be a widespread genetic event. We therefore postulate a direct connection between tumour suppressor networks and miRNA biogenesis machineries. We first compared miRNAs induced by DNA damage and/or p53 activation[15–17], and also p68/p72-regulated miRNAs at the processing steps[7], and noticed that p72-dependent miRNAs tend to be upregulated under DNA-damaging conditions (Supplementary Fig. 1). This observation led us to test the involvement of p53 in miRNA processing, in accord with a previous report of a functional interaction between p53 and p68/p72 (ref. 18).

To identify miRNAs potentially regulated by p53, we measured the expression levels of a selected group of miRNAs by quantitative polymerase chain reaction with reverse transcription (qRT–PCR) after treatment with the DNA-damaging agent doxorubicin, a potent p53 inducer, in the *p53* wild-type HCT116 human colon cancer cell line. Consistent with previous findings[15–17] was our observation that exposure to doxorubicin increased not only a known p53 target, miR-34a, but also a set of mature miRNAs such as miR-15a, miR-16-1, miR-23a, miR-26a, miR-103, miR-143, miR-145, miR-203 and miR-206 (Fig. 1a). To determine whether their accumulation is mediated transcriptionally or post-transcriptionally, we measured the expression levels of primary precursor, precursor and mature forms of these miRNAs. miR-34a, a direct transcriptional target of p53, increased at all primary, precursor and mature miRNA levels. In contrast, several other miRNAs, including miR-16-1, miR-143, miR-145 and miR-206, showed no significant change in primary transcripts but did show increased expression of precursor and mature miRNAs; this was confirmed by northern blot analysis (Fig. 1a and Supplementary Fig. 2). Similar results were obtained in two human diploid cell lines, WI-38 and TIG-3, and TOV21G ovarian cancer cells (Supplementary Figs 3 and 4, respectively).

The DNA-damage-induced upregulation of pre-miRNAs and mature miRNAs was diminished in $p53^{-/-}$ HCT116 cells (Fig. 1b). siRNA-mediated p53 knockdown also attenuated these increments of pre-miRNAs and mature miRNAs in p53-competent WI-38 cells and HCT116 cells (Fig. 1c and Supplementary Fig. 5). Further, p68 or p72 knockdown abolished the increase of these miRNAs at the precursor and mature levels (Fig. 1c and Supplementary Fig. 5), indicating that several miRNAs are post-transcriptionally upregulated in a p53-dependent and p68/p72-dependent manner after DNA damage. The miRNAs analysed above have been shown to be decreased in various human cancers[1,8,10,13,19,20], and they decreased the proliferation rate of HCT116 cells (Supplementary Fig. 6a). We further found that novel targets of these miRNAs include important regulators of the cell cycle and cell proliferation, such as *K-Ras* (as a target of miR-143) and *CDK6* (as a target of miR-16, miR-26a, miR-107, miR-145 and miR-206) (Supplementary Fig. 6b). Each miRNA suppressed the expression of luciferase reporter gene fused to the fragments of 3′ untranslated regions (3′ UTRs) of *K-Ras* and *CDK6*, containing the corresponding potential target sites (Supplementary Figs 7 and 8). Moreover, luciferase activity was significantly decreased in response to DNA damage (Supplementary Fig. 9). Thus, p53/p68-dependent regulation of several miRNAs might support a growth inhibitory function of p53.

We next investigated the molecular interaction between the Drosha complex and p53, because p53 has previously been shown to interact with p68 (ref. 18). Ectopically expressed Drosha and p53 were associated in HCT116 cells (Fig. 2a). We also found that p53 accumulated and interacted with endogenous Drosha/DGCR8 on

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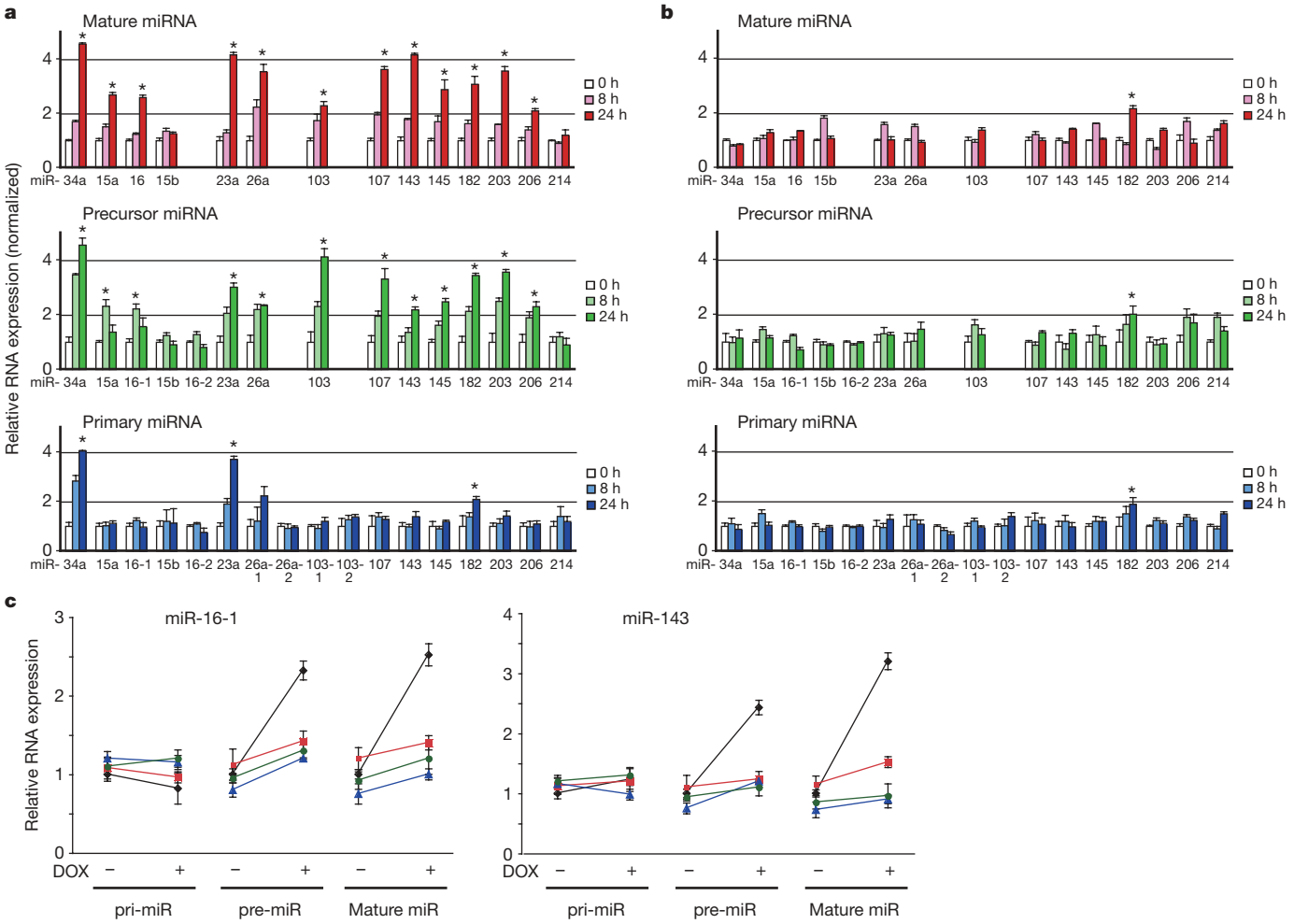

Figure 1 | Post-transcriptional modification of miRNA biogenesis by p53 and p68/p72 in response to DNA damage. **a**, **b**, The expression levels of primary precursor, precursor and mature forms of the indicated miRNAs were examined in *p53* wild-type ($p53^{WT}$) HCT116 cells (**a**) and $p53^{-/-}$ HCT116 cells (**b**) after treatment for 8 or 24 h with 0.2 μg ml^{-1} doxorubicin (asterisk, $P < 0.05$ compared with no treatment; $n = 3$). Error bars represent s.e.m. **c**, Effects of p53, p68 or p72 knockdown on miRNA elevation after treatment with doxorubicin (DOX). WI-38 human diploid fibroblasts were transfected with control siRNA (black) or siRNA for *p53* (red), *p68* (blue) or *p72* (green). After treatment with doxorubicin, the expression levels of primary, precursor and mature miRNAs were compared by qRT–PCR analysis.

treatment with doxorubicin in HCT116, WI-38, TIG-3 and MCF7 cells (Fig. 2b and Supplementary Fig. 10). The association of Drosha and p53 was markedly decreased by treatment with RNase A1 and on p68/p72 knockdown by siRNA (Fig. 2c, d), indicating that p53 interacts with the Drosha complex through certain RNA species and the p68/p72 RNA helicase(s). In addition, we observed that p53 associates with Drosha or p68 through a carboxy-terminal half of the central DNA-binding domain (Supplementary Figs 11 and 12).

In the process of DNA-damage-mediated induction of pre-miRNAs and mature miRNAs, the transcription rates of pri-miR-16-1 and miR-143 were constant in nuclear run-on assay (Fig. 3a) and the levels of unprocessed pri-miRNA tended to decrease (Supplementary Fig. 13). Therefore, on the basis of an interaction between Drosha and p53, we addressed whether p53 modulates the *in vivo* association

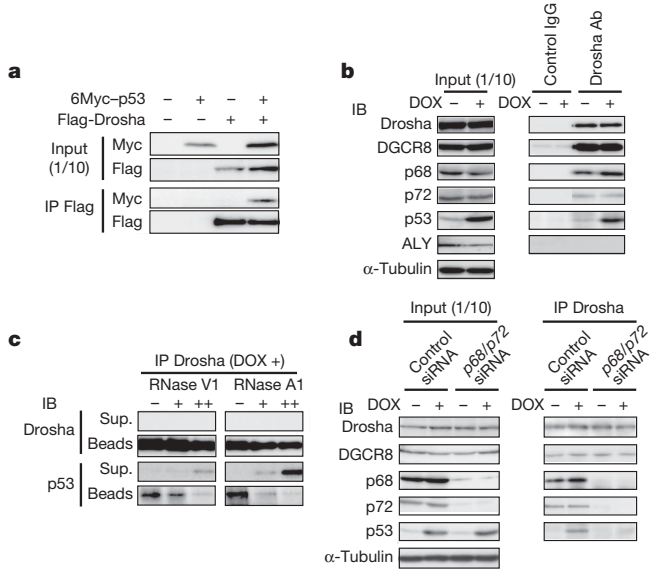

Figure 2 | Association between p53 and Drosha complex. **a**, **b**, Immunoprecipitation assays were performed to detect interaction between ectopic (**a**) and endogenous (**b**) p53 and Drosha in HCT116 cells. An association between endogenous p53 and Drosha was observed under DNA-damaging conditions (**b**). ALY served as a negative control for the immunoprecipitation assay. Ab, antibody; DOX, doxorubicin; IB, immunoblot; IP, immunoprecipitation. **c**, RNA dependence of interaction between p53 and Drosha. Immunoprecipitates of Drosha prepared from HCT116 cells treated for 8 h with doxorubicin were digested with RNase V1 (nuclease for double-stranded RNA and double-stranded DNA) or RNase A1 (RNase for single-stranded RNA), separated to supernatant (sup.) and beads, and subjected to immunoblot analysis. **d**, Immunoblot analysis of Drosha immunoprecipitates from HCT116 cells transfected with control or *p68* and *p72* siRNA, followed by treatment for 8 h with doxorubicin.

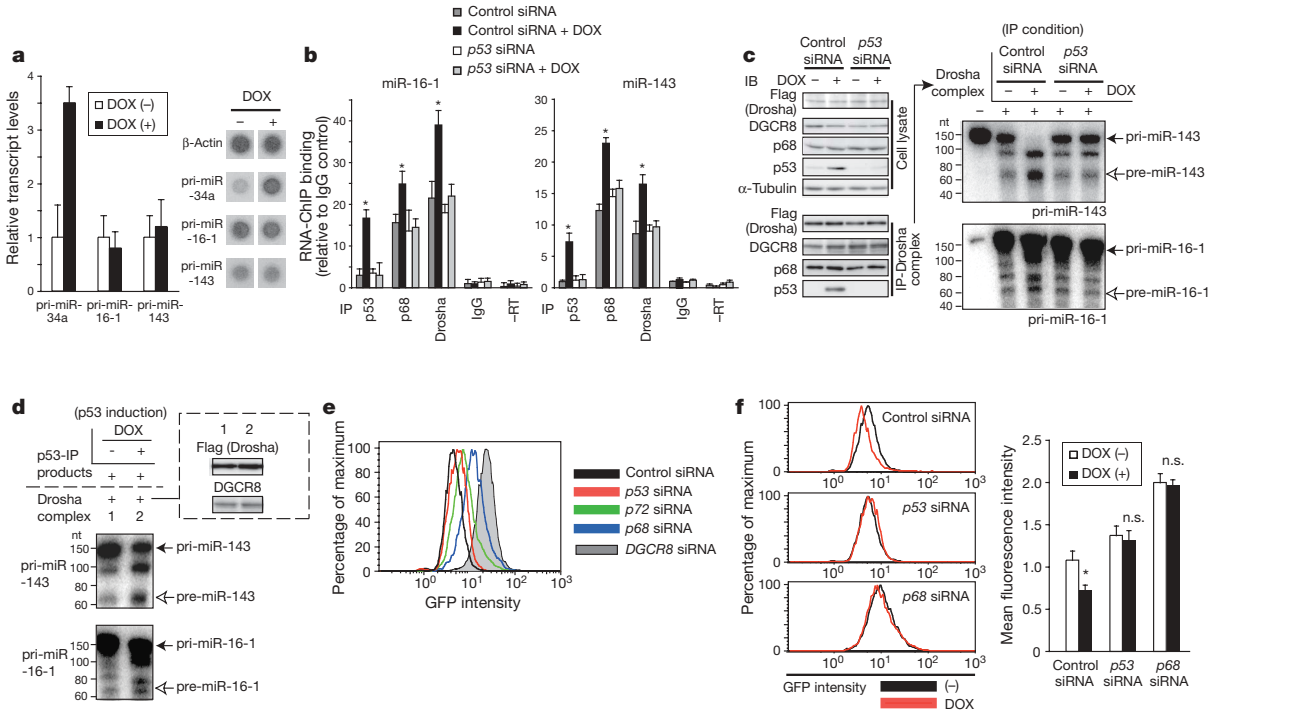

Figure 3 | p53 facilitates Drosha-mediated pri-miRNA processing. **a**, Nuclear run-on assay. After treatment with doxorubicin (DOX), the transcription rates of pri-miR-34a, miR-16-1 and miR-143 were measured and normalized against β-actin levels ($n = 3$). Representative dot-blots are shown in the right panel. **b**, RNA-ChIP analysis for association between pri-miR-16-1/pri-miR-143 and p53, p68 or Drosha in WI-38 cells. After transfection with control or *p53* siRNA, WI-38 cells were treated for 8 h with or without doxorubicin, and endogenous p53, p68 or Drosha were immunoprecipitated and subjected to RT–PCR analysis. As controls, RNA samples untreated with reverse transcriptase (−RT) or immunoprecipitated with non-specific IgG (IgG) were subjected to PCR (asterisk, $P < 0.05$ compared with control siRNA (DOX (−)); $n = 3$). **c**, *In vitro* pri-miRNA processing assay of pri-miR-16-1/pri-miR-143 with immunoprecipitated Flag–Drosha complex from HCT116 cells after siRNA transfection and treatment with doxorubicin. Filled and open arrows indicate the precursor and processed RNAs, respectively. The left panel shows an immunoblot of whole cell lysates and immunoprecipitated Flag–Drosha complex of each sample used for the *in vitro* processing assay. nt, nucleotides. **d**, Addition of immunoprecipitated p53 from HCT116 cells treated for 8 h with doxorubicin facilitated the cleavage of pri-miR-16-1/pri-miR-143 by the Flag–Drosha complex. The top right panel shows an immunoblot of immunoprecipitated Drosha. **e**, **f**, Fluorescence-activated cell sorting analysis of HCT116 cells carrying fusion genes encoding the EGFP cDNA and pri-miR-143/pri-miR-145 (pMXs-puro-EGFP-miR-145/miR-143). DGCR8 or p68 knockdown strongly augmented GFP signals (**e**). Changes in GFP signals were measured after treatment with doxorubicin under p53 or p68 knockdown (**f**). Fluorescence intensities are shown as means and s.e.m.

of pri-miRNAs with the Drosha complex and Drosha function. RNA-chromatin immunoprecipitation (ChIP) analysis showed that p53 associates with pri-miR-16-1/pri-miR-143 after treatment with doxorubicin (Fig. 3b and Supplementary Fig. 14). Treatment with doxorubicin also significantly enhanced the association between these pri-miRNAs and Drosha or p68 in a p53-dependent manner, in both WI-38 cells and HCT116 cells (Fig. 3b and Supplementary Fig. 14), suggesting that the p53 pathway promotes the recruitment of the p68–Drosha complex to the target pri-miRNAs.

We also performed an *in vitro* pri-miRNA processing assay by incubating radiolabelled pri-miR-16-1/pri-miR-143 substrate with immunoprecipitated Flag-tagged Drosha complex from HCT116 cells treated with or without doxorubicin. Drosha complex from doxorubicin-treated cells contained higher pri-miRNA processing activity than that from mock-treated cells, and the higher processing activity was abolished by knockdown of p53 (Fig. 3c). Furthermore, an *in vitro* combination of immunoprecipitated p53 and Drosha complex reconstructed the facilitation of pri-miRNA processing (Fig. 3d). We next established *in vivo* cellular systems for convenient and real-time monitoring of Drosha function[21]. HCT116 cells were infected with retroviral constructs carrying fusion genes encoding enhanced green fluorescent protein (EGFP) and a short segment of pri-miR-143/pri-miR-145. We predicted that the EGFP transcripts should lose their poly(A) tail by Drosha-mediated cleavage of pri-miRNAs, resulting in their poor stability and decreased translation. In this cell line, GFP intensity was inversely correlated with Drosha activity (Supplementary Fig. 15). DGCR8 or p68 knockdown strongly enhanced the GFP signal, whereas p72 knockdown modestly enhanced it (Fig. 3e). In this system we observed that DNA damage by doxorubicin caused a decrease in GFP signal; this attenuation was decreased by knockdown of p53 or p68 (Fig. 3f). Taken together, these results demonstrate that p53 enhances Drosha-mediated processing of several miRNAs both *in vitro* and *in vivo* under DNA-damage-inducing conditions.

p53 mutations are frequently observed in human tumours, and most of these have missense mutations that lead to a loss of wild-type p53 transcriptional activity and to the accumulation of stable p53 mutants in cancer cells[22]. Some mutant p53 proteins such as R175H and R273H have been shown to exert oncogenic activities in addition to a loss of function[23–25]. We therefore examined the effects of several tumour-derived transcriptionally inactive p53 mutants (C135Y, R175H and R273H) on miRNA processing through their introduction into *p53*-null HCT116 cells. The p53 mutants suppressed the precursor and mature miRNA levels of miR-16-1, miR-143 and miR-206, in comparison with the constant level of pri-miRNAs, whereas wild-type p53 increased the pre-miRNA and mature miRNA expression levels of these miRNAs (Fig. 4a–c). The mutant p53 also decreased the production of mature and precursor miR-16-1 and miR-143 from the ectopically expressed pri-miR-16-1/pri-miR-143 (Supplementary Figs 16 and 17), suggesting that the mutant p53 hinders miRNA processing in a transcription-independent manner. We verified that the p53 mutants heightened the GFP signals as well as p68 knockdown in the *in vivo* monitoring of microprocessor activity (Fig. 4d and Supplementary Fig. 18). To elucidate

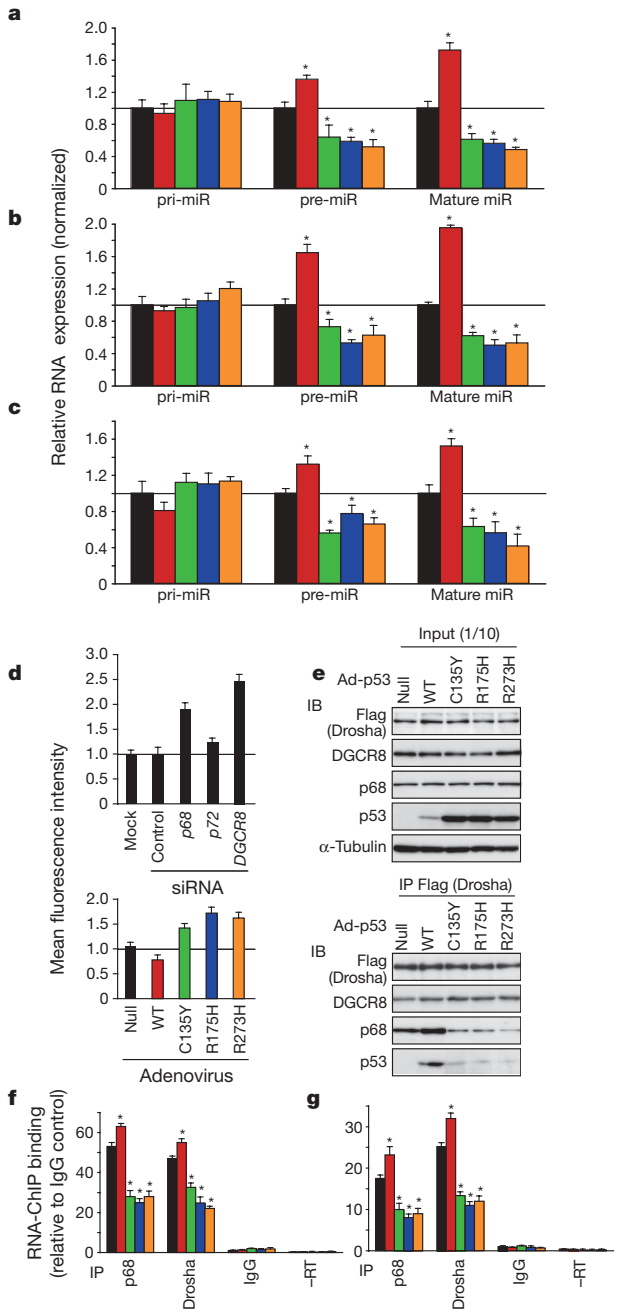

Figure 4 | Deregulation of miRNA processing by mutant p53. a–c, $p53^{-/-}$ HCT116 cells were infected with adenovirus carrying wild-type (red) or tumour-derived transcriptionally inactive mutant p53 (C135Y (green), R175H (blue) and R273H (orange)) or control adenovirus (Ad-null; black). The amounts of primary precursor, precursor and mature forms of miR-16-1 (**a**), miR-143 (**b**) and miR-206 (**c**) were examined (asterisk, $P < 0.05$ compared with Ad-null; $n = 3$). Error bars represent s.e.m. **d**, Fluorescence-activated cell sorting analysis of $p53^{-/-}$ HCT116 cells harbouring pMXs-puro-EGFP-miR-145/miR-143. Mean fluorescence intensities of GFP signals were measured after infection with Ad-p53 (wild-type (WT), C135Y, R175H and R273H) or transfection with siRNAs. Error bars represent s.e.m. **e**, Immunoprecipitation (IP) assays were performed after infection with Ad-p53 (WT, C135Y, R175H and R273H) or Ad-null. IB, immunoblotting. **f**, **g**, RNA-ChIP analysis. After infection of $p53^{-/-}$ HCT116 cells with Ad-p53, endogenous proteins were immunoprecipitated by anti-p68 or anti-Drosha antibodies and subjected to RT–PCR analysis with miR-16-1 (**f**) or miR-143 (**g**) primers (asterisk, $P < 0.05$ compared with Ad-null; $n = 3$). Colour coding is as in **a–c**.

the mechanisms of decreased miRNA processing by the p53 mutants, we examined the effect of mutant p53 expression on the association between Drosha and p68. As shown in Fig. 4e, the accumulation of mutant p53 decreased the interaction between Drosha and p68, but p53 mutants still interacted with p68 (data not shown). In contrast, wild-type p53 expression induced a modest increase in the Drosha–p68 association (Fig. 4e). Finally, RNA-ChIP analysis also revealed that the mutant p53 decreased the association between pri-miR-16-1/pri-miR-143 and p68 or Drosha (Fig. 4f, g). Tumour-derived p53 mutations might therefore endow some different and opposite properties on this protein, leading to interference with miRNA biogenesis by negative titration of Drosha microprocessor components and interference with Drosha/p68 accessibility to several pri-miRNAs. In support of this notion, widespread downregulation of miRNA was prominent in late-stage or high-grade ovarian cancers with a higher frequency of p53 mutation and overexpression[9].

This study raises a possibility that p53 functions as a global modifier of gene expression through the regulation of miRNA processing. The production of mRNA isoforms with a different 3′ UTR, a major docking platform for miRNAs, is regulated by alternative polyadenylation. It has recently been shown that increased proliferation is coupled with widespread decrease in lengths and miRNA target site abundance in 3′ UTRs of mRNA[26]. Consistently, avoidance of strict gene regulation by miRNAs might be a general feature of cancer cells. Thus, a miRNA-controlled gene regulatory layer may itself serve as a part of global program for cellular proliferation and have an intrinsic tumour-suppressive function in conjunction with the p53 network. In addition, a loss of mature miRNAs has been shown to result in increased DNA damage and p53 activity, indicating a reciprocal connection between the p53 and miRNA pathways[27].

Apart from the important role of p53 as a sequence-specific transcription factor, previous studies have revealed that its transcription-independent roles contribute to the regulation of apoptosis and to DNA repair and recombination[28,29]. Here we have shown an unexpected function of p53 in miRNA processing. Further investigation would provide insights into how great a portion of pri-miRNAs are regulated by p53 and how the specificities of recognition of pri-miRNAs are determined. A recent study also demonstrated a similar regulatory pathway of miRNA biosynthesis mediated by nuclear SMAD proteins[30], indicating intimate crosstalks between miRNA-processing machineries and nuclear factors. The present study offers an insight into a new approach to cancer treatment by modulating the p53 and miRNA pathways.

METHODS SUMMARY

Cell lines. The human colon cancer cell line HCT116 and isogenic $p53^{-/-}$ HCT116 cells were provided by B. Vogelstein. Two human diploid fibroblast lines, WI-38 and TIG-3, were obtained from the RIKEN Cell Bank and the Health Science Research Resources Bank, respectively. The TOV21G human ovarian cancer cell line was purchased from the American Type Culture Collection.

qRT–PCR analysis. qRT–PCR assays were performed for measurement of the expression levels of primary, precursor and mature miRNAs as described previously[30].

RNA-ChIP. RNA-ChIP was performed as described previously[7].

***In vitro* pri-miRNA processing analysis.** The *in vitro* pri-miRNA processing assay was performed as described previously[6,7]. *In vitro* transcription was conducted with linearized pcDNA3 vector containing pri-miRNA. The processing reaction, containing the immunoprecipitated Drosha complex (with/without immunoprecipitated p53) and the internally [α-^{32}P]UTP-labelled pri-miRNA was incubated at 37 °C for 90 min.

***In vivo* monitoring of pri-miRNA processing.** Retroviral vectors with fusion genes of the EGFP cDNA and pri-miRNA[21] were introduced into HCT116 cells. High-responder clones to DGCR8 knockdown were selected. The change in GFP signals was measured by flow cytometric analysis.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions H.I.S. conceived and designed the research, performed experiments and analyses and wrote the paper. Y.K., T.I. and S.K. provided key materials. K.S. and K.M. supervised the whole project and wrote the paper.

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METHODS

Antibodies. The following antibodies were used: Flag epitope tag M2 (Sigma); Myc epitope tag 9E10 (Pharmingen) or 71D10 (no. 2278) (Cell Signaling), p53 DO-1 (sc-126) or FL-393 (sc-6243) (Santa Cruz), p68 05-850 (Upstate), p72 ab24601 (Abcam), CDK6 B-10 (sc-7961) (Santa Cruz), K-Ras F234 (sc-30) (Santa Cruz), α-tubulin DM-1A (Sigma), DGCR8 10996-1-AP (Proteintech), Drosha 07-717 (Upstate) and ALY 11G5 (sc-32311) (Santa Cruz).

qRT–PCR assays and northern blot analysis. qRT–PCR assays were performed for measurement of the expression levels of primary, precursor and mature miRNAs, as described previously[30]. In brief, total RNA was extracted with Trizol (Invitrogen) and subjected to reverse transcription with the Quantitect Reverse Transcription kit (Qiagen) according to the manufacturer's instructions. qRT–PCR was performed with the 7500 Fast Real-Time PCR System (Applied Biosystems). For detection of mature miRNAs, TaqMan MicroRNA assay kit (Applied Biosystems) was used in accordance with the manufacturer's protocol. Data analysis was performed by using the comparative C_t method. Results were normalized to β-actin or U6 snRNA. The expression ratio of U6 snRNA and β-actin were constant on treatment with doxorubicin. For measurement of the precursor miRNA expression, as an alternative approach, small RNA fraction was purified with the miRvana miRNA isolation kit (Ambion) and subjected to reverse transcription and qRT–PCR analyses (normalization by U6 snRNA); similar results were obtained by this method. The primer sequences used are given in the Supplementary Information. Northern blot analysis was performed as described previously[6,7]. Oligonucleotides complementary to miRNAs were end-labelled with [γ-^{32}P]ATP and used as probes for northern analysis.

siRNAs. The siRNAs (except that for *DGCR8*) and control siRNA (ON-TARGET plus non-targeting pool) were purchased from Dharmacon (SMARTpool). siRNA for *DGCR8* and control siRNA were purchased from Invitrogen (Stealth RNAi). siRNAs (20 nM) were introduced into cultured cells with HiPerfect transfection reagent (Qiagen). The transfected cells were used for subsequent experiments after 24–48 h. The target sequences of these siRNA duplexes are given in Supplementary Information.

Construction of p53 deletion vectors. p53 deletion constructs (ΔTAD (Δ1–45), ΔPRD (Δ62–91), ΔDBD (Δ114–290), ΔDBD-N (Δ114–212), ΔDBD-C (Δ213–290) and ΔC (Δ291–393)) were prepared as described in previous reports[31–34].

Adenovirus. Wild-type and mutant p53 (C135Y, R175H and R273H) cDNAs were cloned into a pENTR vector (Invitrogen) and introduced into the pAd/CMV/V5-DEST vector by using LR Clonase (Invitrogen). HEK293A cells were transfected with pAd/CMV/p53 (WT/C135Y/R175H/R273H) after linearization with *Pac*I. Viral particles were isolated by three freeze–thaw cycles and amplified by reinfection to HEK293A cells.

Lentivirus. HCT116 cells stably expressing Flag–Drosha were generated by lentiviral infection system (a gift from H. Miyoshi). Flag–*Drosha* cDNA was transferred into lentivirus vector (CSII-CMV-RfA) by means of a pENTR vector, with LR clonase. The lentivirus production was carried by transfection of HEK293FT cells with vector construct, pCMV-VSV-G-RSV-Rev and pCAG-HIVgp. The culture supernatants were collected 48 h after transfection, and viral particles were introduced to HCT116 cells.

Immunoprecipitation and immunoblot assay. Cells were lysed with a buffer containing 1% Nonidet P40, 20 mM Tris-HCl pH 7.4, 150 mM NaCl, 5 mM EDTA and 1% protease inhibitor mixture (Nacalai Tesque). For immunoprecipitation, cleared lysates were incubated with the indicated antibodies. For RNase V1 (nuclease for dsRNA/dsDNA) and RNase A1 (RNase for ssRNA) digestion, the immunoprecipitated proteins were washed twice with RNase digestion buffer (Ambion) and suspended in the same buffer containing RNase V1 or A1 (Ambion). The samples were incubated at 37 °C for 30 min and quickly separated by centrifugation into two fractions: beads containing the still-bound proteins and supernatant containing the released proteins. Total cell lysates or proteins in immunoprecipitates were subjected to SDS–PAGE and transferred to Fluoro Trans W membrane (Pall). Immunoblotting was performed with the indicated antibodies.

Proliferation and cell cycle analysis. Pre-miR miRNA Precursors were purchased from Ambion. For growth experiments, cells were seeded in 96-well plates and transfected with pre-miR precursors (20 nM), and growth rates were measured with a WST-8 assay kit (Nacalai Tesque). The cell cycle distribution was determined with a FACScan flow cytometer (Beckman Coulter) using CycleTEST PLUS DNA Reagent kit (BD Biosciences).

UTR reporter assay. The 3′ UTR segments of *K-Ras* and *CDK6* were cloned into the Psicheck 2 dual luciferase reporter vector (Promega). HCT116 cells were then transfected with each reporter construct with and without pre-miR precursors. Cell extracts were prepared 24–48 h after transfection, and the ratio of *Renilla* to firefly luciferase was measured with the Dual-Luciferase Reporter Assay System (Promega). The primer sequences used for cloning of the 3′ UTR segments are given in the Supplementary Information.

RNA-ChIP. RNA-ChIP was performed as described previously[7,35]. All the buffers used in this study contained 0.5 U μl^{-1} RNase inhibitor (Toyobo). Nuclei from HCT116 cells were first isolated from 1% formaldehyde-fixed cells and used for chromatin fragmentation. After immunoprecipitation with anti-p53, anti-p68 or anti-Drosha antibody, washing and elution, the precipitated RNA–DNA pellets were resuspended in 100 μl of nuclease-free water containing 1 μl of 40 U μl^{-1} RNase inhibitor, 5 μl of 1 M Tris-HCl pH 7.5, 20 μl of 50 mM $MgCl_2$ and 4 μl of 10 U μl^{-1} DNase I. The mixture was incubated for 30 min at 37 °C and extracted once with phenol/chloroform (5:1). RNA was precipitated with ethanol and dissolved in 30 μl of nuclease-free water. A 29-μl aliquot of the RNA was used for a 60-μl cDNA synthesis reaction and qRT–PCR analysis. The primer sequences used are given in Supplementary Information.

Nuclear run-on assay. The nuclear run-on assay was performed as described previously[36]. An aliquot (0.5 μg) of each PCR fragment amplified by primers for the qRT–PCR assay was dot-blotted onto positively charged nylon membranes (Roche). After isolation of the nucleus and run-on transcription reaction, RNA probe was prepared with Trizol reagent. Membranes were probed with ^{32}P-labelled run-on RNA, and then analysed with a phosphorimager.

***In vitro* pri-miRNA processing analysis.** *In vitro* pri-miRNA processing assay was performed as described previously[6,7,37]. *In vitro* transcription was performed with linearized pcDNA3 vector containing pri-miR-16-1, pri-miR-143 or pri-miR-145. The processing reaction, containing the immunoprecipitated Drosha complex from HCT116 cells (with or without immunoprecipitated p53 from HCT116 cells), 3 μl of a solution containing 32 mM $MgCl_2$, 10 mM ATP, 200 mM creatine phosphate, 20 U μl^{-1} RNase inhibitor, internally [α-^{32}P]UTP-labelled pri-miRNA and buffer (20 mM Tris-HCl pH 7.9, 0.1 M KCl, 10% glycerol, 5 mM dithiothreitol, 0.2 mM phenylmethylsulphonyl fluoride) was added to a final volume of 30 μl. The reaction mixture was incubated at 37 °C for 90 min and extracted with phenol/chloroform, then with chloroform, and precipitated with glycogen and ethanol. The precipitated RNA was loaded on 15% denaturing polyacrylamide gels, and then autoradiographed.

***In vivo* monitoring of pri-miRNA processing.** Retroviral constructs with fusion genes of the EGFP cDNA and pri-miRNA (pMXs-puro-miR-143-IRES-EGFP, pMXs-puro-EGFP-miR-145/miR-143 and pMXs-puro-chimaera-miR-143-EGFP) were described previously[21]. A retroviral supernatant was obtained by transfection of G3T-hi cells by using a Retrovirus Constructive Kit Ampho (Takara Bio) in accordance with the manufacturer's instructions. Retroviral infection was performed with standard procedures, and the cells were selected with 1 μg ml^{-1} puromycin, 48 h after infection. High-responder clones to DGCR8 knockdown were selected. After treatment with doxorubicin, transfection with siRNA or infection with adenovirus, cells were analysed with a FACScan flow cytometer (Beckman Coulter) and the data were analysed with Flowjo (Tree Star).

Statistical analysis. Statistical differences were determined by analysis of variance followed by post-hoc comparison with the Fisher's protected least-significant-difference test. Statistical significance is shown as $P < 0.05$ (asterisk).

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