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MicroRNA-7 downregulates XIAP expression to suppress cell growth and promote apoptosis in cervical cancer cells

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ABSTRACT

Our study demonstrated the functions of microRNA-7 (miR-7) in cervical cancer. The overexpression of miR-7 in the cervical cancer cell lines HeLa and C-33A suppressed cell viability and promoted cell apoptosis, whereas the inhibition of miR-7 had opposite effects. Furthermore, an oncogene, X-linked inhibitor of apoptosis protein (XIAP), was identified as a new target of miR-7, and the ectopic expression of XIAP rescued the effects induced by miR-7 in HeLa and C-33A cells. These results indicate that miR-7 targeted and downregulated the oncogene XIAP to regulate the effect of miR-7 on apoptosis and malignant behaviors of HeLa and C-33A cells.

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1. Introduction

MicroRNAs (miRNAs) are a group of small, non-coding RNA molecules, approximately 19–25 nucleotides in length, that regulate gene expression by targeting mRNAs to trigger either translational repression or mRNA degradation [1,2]. miRNAs are involved in cellular processes such as development, proliferation, apoptosis, stress response, etc. [3,4]. Documented evidence has shown that the altered expression of specific miRNA genes contributes to the initiation and progression of cancer [2]. For instance, miR-218 [5], miR-10a [6] and miR-181b [7] acts as tumor suppressors in medulloblastoma, hepatoma cells and gastric adenocarcinomas, respectively. Studies on the function and mechanism of miRNAs can be especially useful in improving cancer treatments.

Cervical cancer is the second leading cause of cancer mortality in women worldwide [8]. Previous reports from other labs and our lab have shown that many miRNAs are aberrantly expressed in cervical cancer and that miRNAs can act as tumor suppressors (e.g., miR-302–367, miR-424, miR-214) [9–12] or enhancers (e.g., miR-205, miR-21, miR-19a/b) [13–15]. Although some progress has been achieved in the past decades, much more effort is needed to elucidate the occurrence and mechanism of cervical cancer.

Extensive research has shown that microRNA-7 (miR-7) was deregulated and played a tumor suppressor role in many tumors, including hepatocellular carcinoma, lung cancer, and breast cancer, etc. [16–18], but the role of miR-7 in cervical cancer cells remains unclear. According to a bioinformatic prediction, X-linked inhibitor of apoptosis protein (XIAP) is a candidate target gene of miR-7 and has an anti-apoptotic effect, which may mediate miR-7 effection in cervical cancer cells. Thus, we chose XIAP for further study. XIAP has been demonstrated to be highly expressed in several malignancies, including cervical cancer, and plays an important role in regulating both apoptosis and cell proliferation [19–22]. The purpose of this study was to determine the effect of miR-7 on the malignant behaviors of cervical cancer cells and to explore the possible mechanisms of the XIAP gene regulation by miR-7 in cervical cancer cells. These findings may be useful for the development of possible approaches for cervical cancer treatment in a clinical application.

2. Materials and methods

2.1. miRNA target prediction

TargetScan, PicTar, and miRBase were used to predict the putative targets of miR-7.

Abbreviations: miRNA, microRNA; miR-7, microRNA-7; XIAP, X-linked inhibitor of apoptosis protein; ASO, antisense oligonucleotide; EGFP, enhanced green fluorescence protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; UTR, untranslated region; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling

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2.2. Cell culture and transfection

The human cervical cancer cell lines HeLa and C-33A were maintained in RPMI1640 (Invitrogen, Carlsbad, CA) with 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin in a humidified 5% (v/v) atmosphere of CO_2 at 37 °C. Transfection was performed with Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol.

2.3. Cervical cancer tissue, RNA preparation and quantitative RT-PCR analysis

15 pairs of cervical cancer and adjacent normal tissues, were obtained at the Cancer Center of Sun Yat-sen University of Medical Science in accordance with the ethical standards of the institutional committee.

Large and small RNAs from tissue were isolated with the mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) according to the manufacturer's protocol. Total RNAs of cell lines were extracted with Trizol reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. Five micrograms of an RNA sample was reverse transcribed to cDNA using oligo (dT) primers and M-MLV reverse transcriptase (Promega, Madison WI); the cDNA was used for the amplification of XIAP and β-actin. Quantitative RT-PCR (qRT-PCR) was performed to detect the relative transcript levels of miR-7 and XIAP. PCR was performed under the following conditions: 94 °C for 4 min followed by 40 cycles of 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min. The relative expression levels of the gene of interest were calculated by the $2^{-\triangle\triangle Ct}$ method. All primers were synthesized by AuGCT Inc. (Beijing, China).

2.4. Plasmid construction and oligonucleotides

In this study, the following vectors were constructed: pcDNA3/pri-miR-7, pcDNA3/enhanced green fluorescence protein (EGFP)-XIAP-3′UTR, pcDNA3/EGFP-XIAP-3′UTR mutant, pSIH1-H1-copGFP/shRNA-XIAP (shRNA-XIAP) and pcDNA3/XIAP. All the primers and oligonucleotides used in this work are listed in Table 1. We also commercially synthesized a 2′-*O*-methyl-modified antisense oligonucleotide (ASO-miR-7) to perform miR-7 loss of function.

2.5. Western blot

Cultured cells were lysed in RIPA buffer, and lysates were analyzed by a standard western blot procedure [25]. Glyceraldehyde-3-phos-phate dehydrogenase (GAPDH) was used as an endogenous normalizer. The anti-GAPDH antibody, anti-XIAP antibody and horseradish peroxidase (HRP)-conjugated secondary antibody were from Saierbio (Tianjin, China).

2.6. Fluorescent reporter assay

HeLa cells or C-33A cells were cotransfected with pcDNA3/pri-miR-7 or ASO-miR-7 in a 48-well plate followed by the pcDNA3/EGFP-XIAP-3′UTR or pcDNA3/EGFP-XIAP-3′UTR mut. pcDNA3 or ASO-NC was used as the control group. A separate RFP expression vector, pDsRed2-N1 (Clontech, Mountain View, CA), was used for normalization. The cells were lysed 72 h later, and the proteins were harvested. The EGFP and RFP fluorescence intensities were detected with the F-4500 Fluorescence Spectrophotometer (Hitachi, Tokyo, Japan).

2.7. Cell viability assay

Cells were seeded in 96-well plates at 6000 cells per well (HeLa cells) or 8000 cells per well (C-33A cells), 24 h after transfection. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was used to determine cell viability 24, 48, and 72 h after the cells were seeded. The absorbance at 570 nm was measured using an lQuant Universal Microplate Spectrophotometer (BioTek, Winooski, VT).

2.8. Colony formation assay

Colony formation assay was performed according to the methods described previously with HeLa cells and C-33A cells [25]. Colony formation was quantified using the colony formation number.

2.9. TUNEL assay

HeLa cells were transfected with pcDNA3/pri-miR-7 or ASO-miR-7, pcDNA3/XIAP or pSIH1-H1-copGFP/shRNA-XIAP. The cells were seeded on a 14-well slide 18–24 h after transfection. 12–16 h after seeding, the cells were induced by 1 ppc (54 μg/ml) paclitaxel for 8–18 h. Cell apoptosis was detected using the In situ Cell Death Detection Kit and Fluorescein (Roche Applied Science, IN, USA) according to the manufacturer's instructions. 4′,6-diamidino-2-phenylindole (DAPI) staining was used to determine the number of nuclei and the total cell number.

Table 1
Primers and oligonucleotides used in this work.

Name	Sequence (5′–3′)
pri-miR-7-S	5′-CGAGGATCCAAACTGCTGCCAAAACCAC-3′
pri-miR-7-AS	5′-CGGAATTCGTAAATCGGACATTAGTAGAACAG-3′
ASO-miR-7	5′-ACAACAAAAUCACUAGUCUUCCA-3'
ASO-NC	5′-CAGUACUUUUGUGUAGUACAA-3'
XIAP–3′UTR-top	5′-AATTC AATATAAAATA TGTCT CAGATCTTCCAA GCTTC-3'
XIAP–3′UTR-bot	5′-TCGAGAAGC TTGGAAGATCTGAGACATATTTTATATT G-3'
XIAP-3′UTR mut-top	5′-AATTC AATATAAAATATGTCTCAGATGAAGGAA GCTTC-3'
XIAP-3′UTR mut-bot	5′-TCGAGAAGC TTCCTTCATCTGAGACATATTTTATATT G-3'
XIAP-S	5′-GCGAATTCGCCACCATGACTTTTAACAGTTTTGAAG-3′
XIAP-AS	5′-GCAGCCTCGAGGCAGACATAAAAATTTTTTGCTTG-3′
shR-XIAP-top	5′-GATCCTAGGTGAAGGTGATAAAGTAACTCGAGTTACTTTATCACCTTCACCTATTTTTG-3′
shR-XIAP-bot	5′-AATTCAAAAATAGGTGAAGGTGATAAAGTAACTCGAGTTACTTTATCACCTTCACCTAG-3′
β-Actin-S	5′-CGTGACATTAAGGAGAAGCTG-3′
β-Actin-AS	5′-CTAGAAGCATTTGCGGTGGAC-3′
miR-7 RT primer	5′-GTCGTATCCAGTGCAGGGTCCGAGGTGCACTGGATACGACACAACAA-3′
miR-7 Forward primer	5′-TGGAAGACTAGTGATT-3′
U6 RT primer	5′-GTCGTATCCAGTGCAGGGTCCGAGGTGCACTGGATACGACAAAATATGG-3′
U6 Forward primer	5′-TGCGGGTGCTCGCTTCGGCAGC-3′
Reverse primer	5′-CCAGTGCAGGGTCCGAGGT-3′

2.10. Statistical analysis

Statistical significance was determined using Student's *t*-test. The data are expressed as the means ± S.D. A *P* value less than 0.05 was considered statistically significant. All our experiments were repeated more than three times and each experiment has triple wells. One representative experiment was selected to draw diagrams and data analysis.

3. Results

3.1. miR-7 affects cell viability, colony formation and apoptosis in cervical cancer cells

To determine the effects of miR-7 in vitro, we first used qRT-PCR to validate the expression of miR-7 in HeLa and C-33A cells after transfection with a miR-7 expression vector (pri-miR-7) or miR-7 antisense oligonucleotide (ASO-miR-7). The miR-7 expression level was upregulated in cells transfected with pri-miR-7 and decreased in cells transfected with ASO-miR-7 (Fig. 1A and B). Next, MTT, colony formation and TUNEL assays were performed to examine the effect of miR-7 on HeLa and C-33A cells. The MTT assay showed that upregulated miR-7 reduced cell viability, whereas ASO-miR-7 increased cell viability in HeLa cells (Fig. 1C). The colony formation assay showed that upregulated miR-7 reduced the colony formation number by approximately 40%, whereas ASO-miR-7 increased colony formation number up to approximately 1.5-fold in HeLa cells (Fig. 1D). Similar results were obtained in C-33A cells (Fig. 1E and F). The TUNEL assay showed that the upregulated miR-7 increased the paclitaxel-induced apoptosis in HeLa cells (Fig. 1G), but ASO-miR-7 did not significantly influence cell apoptosis (Fig. 1G), which may be due to the endogenous low expression of miR-7. These results demonstrate that miR-7 can suppress cell growth and induce cell apoptosis in cervical cancer cells.

3.2. miR-7 binds directly to the XIAP 3′UTR in cervical cancer cells

To determine the target gene that mediates the effect of miR-7 on the cervical cancer cells, TargetScan, PicTar, and miRBase were used to predict the potential targets of miR-7. Among these genes, XIAP (GenBank Accession NM_001167.3) was predicted as a potential target of miR-7 and selected as our target gene because of its correlation to apoptosis resistance. We found that the 3′UTR of the XIAP mRNA contains a miR-7-complementary binding site, which is conserved among species (Fig. 2A). EGFP reporter assays were performed with EGFP reporter vectors carrying the XIAP 3′UTR or XIAP 3′UTR mut. In HeLa cells, the overexpression of miR-7 by pri-miR-7 repressed EGFP expression by approximately 20% compared with the control vector (Fig. 2B), whereas the downregulation of miR-7 with ASO-miR-7 increased the intensity of EGFP fluorescence up to approximately 1.35-fold compared with the control group (Fig. 2B). The EGFP expression in HeLa cells transfected with XIAP 3′UTR mut was not affected by the overexpression or knockdown of miR-7 (Fig. 2C). In addition, qRT-PCR and western blot analysis were used to determine whether miR-7 affects endogenous XIAP mRNA and protein levels. In HeLa cells, qRT-PCR indicated the relative transcript levels of XIAP after transfection with pri-miR-7 were downregulated by 50% compared with the control group, but increased up to 1.4-fold after transfection with ASO-miR-7 compared with the control group (Fig. 2D). Western blot showed that the protein expression level of XIAP gene was downregulated or upregulated by the overexpression or knockdown of miR-7 in HeLa cells (Fig. 2E). Similar results were obtained in C-33A cells (Fig. 2B–D, F). In addition, to investigate the expression of miR-7 and XIAP in cervical cancer tissues, qRT-PCR assays were performed on 15 pairs of cervical cancer and adjacent normal tissues to detect the expression of miR-7 and XIAP. Under these conditions, the miR-7 expression level was lower (Fig. 2G) and XIAP expression level was higher (Fig. 2H) in the tumor tissues compared with the matched normal tissues, which indicates that miR-7 and XIAP expressions levels are conversely

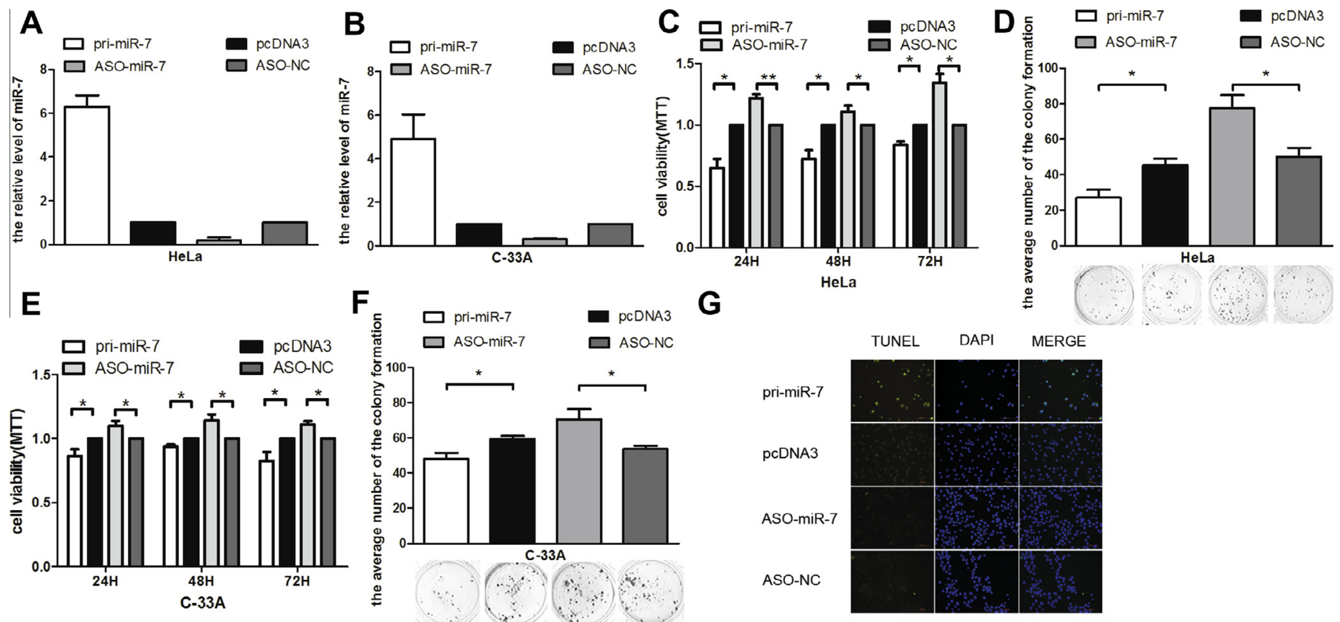

Fig. 1. miR-7 affects cell viability and colony formation in HeLa and C-33A cells in vitro. (A and B) The expression level of miR-7 following the transfection of pri-miR-7 or ASO-miR-7 in HeLa and C-33A cells. U6 snRNA was used for endogenous normalization. (C–F) HeLa or C-33A cells were transfected with either pri-miR-7 or ASO-miR-7. Cell viability was detected by the MTT assay at 24, 48 and 72 h (C and E). HeLa or C-33A cells were transfected with either pri-miR-7 or ASO-miR-7. The cell growth capacity in vitro was assessed by the colony formation assay (D and F). (G) HeLa cells were transfected with pri-miR-7 or ASO-miR-7 and treated with 1 ppc (54 μg/ml) paclitaxel for 18 h. Fluorescein staining indicated apoptotic cells. DAPI staining was used to determine the number of nuclei and to assess the gross cellular morphology. (*$P < 0.05$; **$P < 0.01$).

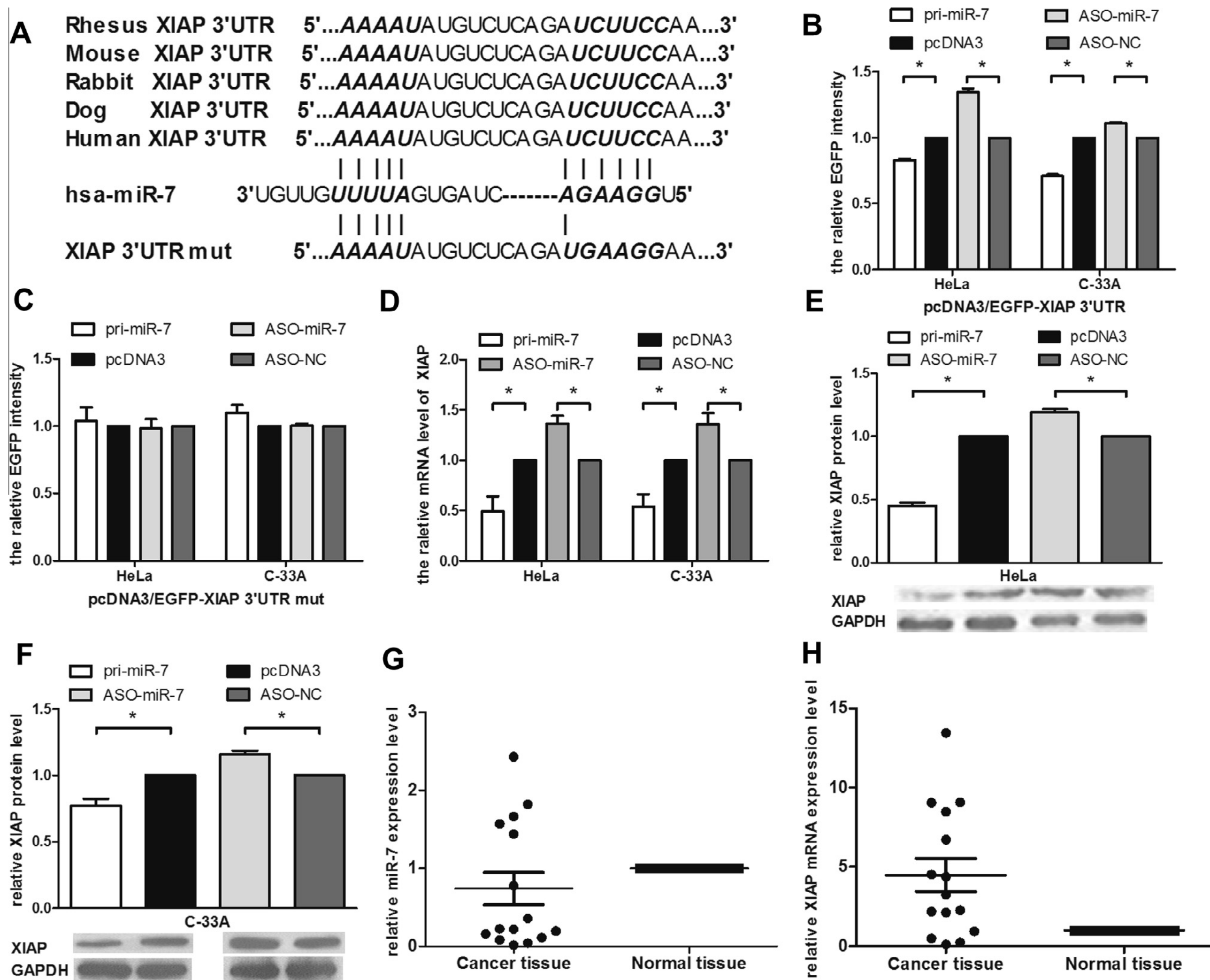

Fig. 2. XIAP is directly repressed by miR-7. (A) The XIAP 3′UTR has a putative miR-7 binding site that is conserved in human, rhesus, mouse, rabbit and dog mRNA. Sequence alignment of miR-7 with the wild-type and mutant (mut) 3′UTR of XIAP. (B) The intensity of EGFP fluorescence in HeLa and C-33A cells transfected with pri-miR-7 was decreased after 48 h and increased following transfection with ASO-miR-7. (C) Pri-miR-7 and ASO-miR-7 had no effect on the intensity of EGFP fluorescence in HeLa and C-33A cells transfected with the 3′UTR mutant vector. (D) The expression level of XIAP in HeLa and C-33A cells was significantly altered following transfection with either pri-miR-7 or ASO-miR-7 as determined by qRT-PCR using U6 snRNA for normalization. (E and F) The protein levels of XIAP in HeLa and C-33A cells decreased or increased compared with the control group when miR-7 was overexpressed or blocked, respectively. (G and H) The relative expressions of miR-7 and XIAP in the 15 pairs of cervical cancer tissues and matched normal tissues were determined with qRT-PCR. U6 snRNA or β-actin was used for normalization. ($^{*}P < 0.05$; $^{**}P < 0.01$).

correlated in cervical cancer tissues. These results suggest that miR-7 directly binds to the XIAP 3′UTR and specifically suppresses the expression of XIAP.

3.3. XIAP increases cell viability, colony formation and suppresses cell apoptosis in vitro

To examine the effect of XIAP on cervical cancer cells, we used gain-of-function and loss-of-function assays. First, we validated the efficiency of a XIAP expression vector, pcDNA3/XIAP, in HeLa cells (Fig. 3A and B). The overexpression of XIAP enhanced cell viability (Fig. 3C) and increased colony formation number up to approximately 1.4-fold (Fig. 3D) compared with control group in HeLa cells. In addition, we suppressed XIAP expression in HeLa and C33A cells with a plasmid expressing a specific small interfering hairpin RNA targeting XIAP (pSIH1-H1-copGFP/shRNA-XIAP, shR-XIAP). qRT-PCR and western blot indicated that shR-XIAP reduced the XIAP mRNA by approximately 43% and protein levels by approximately 54% of the normal level in HeLa cells, respectively (Fig. 3A and B). Furthermore, the inhibition of XIAP expression decreased viability (Fig. 3C) and reduced colony formation by approximately 30% (Fig. 3D) in HeLa cells compared with the control groups. Similar results were obtained in C-33A cells (Fig. 3E–H). The TUNEL assay showed that the knockdown of XIAP promoted cell apoptosis and that the overexpression of XIAP suppressed cell apoptosis in HeLa cells (Fig. 3I). These results demonstrate that the effect of XIAP knockdown on cell proliferation and apoptosis is consistent with the effect of miR-7 overexpression in HeLa and C33A cells.

3.4. Ectopic expression of XIAP counteracts the effects of miR-7 in cervical cancer cells

To further confirm that the effects of miR-7 on the proliferation and apoptosis of cervical carcinoma cells are mediated by XIAP, pcDNA3/XIAP was constructed. This construct contained the coding sequence of XIAP without the 3′UTR to avoid miRNA interference. MTT (Fig. 4A and B), colony formation (Fig. 4C and D) and western blot assays (Fig. 4E and F) showed that the ectopic expression of XIAP rescued the effects caused by miR-7 in HeLa and C-33A

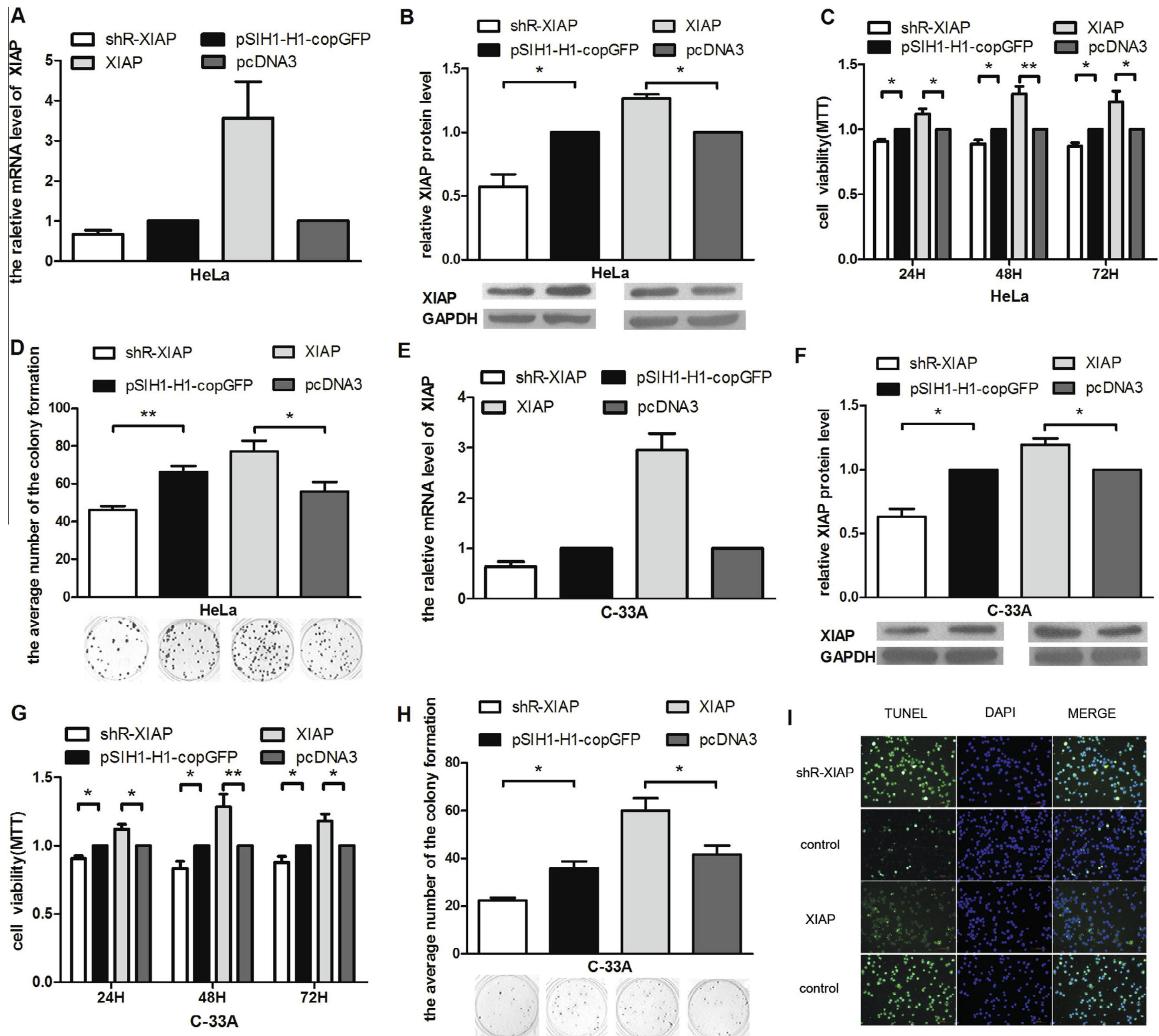

Fig. 3. XIAP affects viability, colony formation and apoptosis in HeLa and C-33A cells. (A–H) The mRNA level of XIAP in HeLa cells or C-33A cells transfected with shR-XIAP or pcDNA3/XIAP (A and E). The protein levels of XIAP in HeLa and C-33A cells was decreased or increased compared with the control group when XIAP was blocked or overexpressed, respectively (B and F). HeLa or C-33A cells were transfected with either shR-XIAP or pcDNA3/XIAP. Cell viability was detected by the MTT assay at 24, 48 and 72 h (C and G). In HeLa and C-33A cells, the colony formation number was lower or higher compared with the control vector after transfected with either shR-XIAP or pcDNA3/XIAP, respectively (D and H). (I) HeLa cells were transfected with shR-XIAP or pcDNA3/XIAP and treated with 1 ppc (54 μg/ml) paclitaxel for 8 h. Fluorescein staining indicated apoptotic cells. DAPI staining was used to determine the number of nuclei and to assess the gross cellular morphology. ($^{*}P < 0.05$; $^{**}P < 0.01$). (In the figure, the pcDNA3/XIAP plasmid was represented by XIAP).

cells. A TUNEL assay (Fig. 4G) also indicated that the overexpression of XIAP could counteract the effect of miR-7 on HeLa cell apoptosis. These results show that miR-7 suppresses cell viability and colony formation and promotes apoptosis by downregulating XIAP expression in cervical carcinoma cells.

4. Discussion

In recent decades, a number of miRNAs have been found and shown to play important roles in gene expression regulation through the alteration of mRNAs or translation [23,24]. Many studies have shown that miRNAs may function as either tumorigenic or tumor-suppressing genes [25,26]. In our study, we found that in cervical cancer cells, miR-7 suppressed cell viability, colony formation and promoted apoptosis in cervical cancer cells through downregulating the expression of XIAP by targeting the 3′UTR of XIAP. In Fig. 1G, ASO-miR-7 did not significantly affect cell apoptosis in HeLa cells; this is possibly due to a low expression level of miR-7 in cervical cancer cells. The low expression of miR-7 in cervical cancer tissues compared with the adjacent non-tumor tissue (Fig. 2G and H) support this notion.

XIAP is involved in regulating a number of functions [27]. Reports showed that XIAP was overexpressed in several classes of cancer [28] and suppressed apoptosis [29]. Our results were consistent with this. Biological prediction and the apoptosis resistance function of XIAP in many tumors helped identify XIAP as target gene of miR-7 [19–22]. In our study, the loss of XIAP function showed growth suppression and apoptosis promotion in HeLa

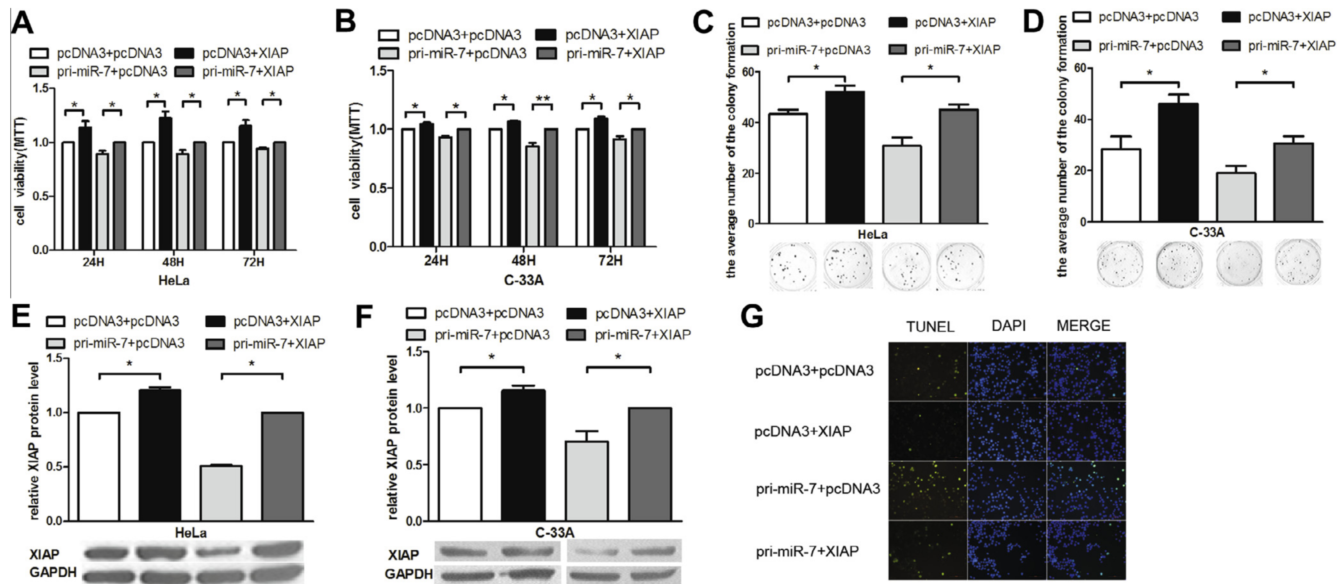

Fig. 4. XIAP rescues miR-7-induced cellular phenotypes in cervical cancer cells. HeLa cells or C-33A cells were co-transfected with the pcDNA3/XIAP vector, which did not contain the 3′-UTR of XIAP, with or without the pri-miR-7 vector. (A and B) Cell viability was detected by the MTT assay at 24, 48 and 72 h after transfection. (C and D) A colony formation assay was performed after transfection. (E and F) The XIAP protein level was measured by western blot 48 h after transfection. (G) The 1 ppc (54 μg/ml) paclitaxel-induced apoptosis of HeLa cells for 12 h was determined by TUNEL assays. ($^{*}P < 0.05$; $^{**}P < 0.01$).

and C-33A cells, which is consistent with the results of miR-7 overexpression. The XIAP gain-of-function assay showed growth promotion and apoptosis suppression in HeLa and C-33A cells, which is consistent with the results of miR-7 knockdown. Ectopic expression of XIAP rescued the effects caused by miR-7. Thus, a series of results support the hypothesis that miR-7 functions in cervical cancer cells by targeting the 3′UTR of XIAP.

In summary, our study shows that miR-7 functions as a tumor suppressor gene and further extends the biological role of miR-7 in HeLa and C-33A cells. For the first time, we identify XIAP as a new target that is possibly involved in miR-7-mediated growth suppression and apoptosis induction of cervical cancer cells. These findings may provide a basic rationale for the application of miR-7 in the treatment of cervical cancer.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2013.05.054.

References

[1] Tang, J., Ahmad, A. and Sarkar, F.H. (2012) The role of MicroRNAs in breast cancer migration, invasion and metastasis. Int. J. Mol. Sci. 13, 13414–13437.
[2] Croce, C.M. and Calin, G.A. (2005) MiRNAs, Cancer, and Stem Cell Division. Cell 122, 6–7.
[3] Cui, F., Li, X., Zhu, X., Huang, L., Huang, Y., et al. (2012) MiR-125b inhibits tumor growth and promotes apoptosis of cervical cancer cells by targeting phosphoinositide 3-kinase catalytic subunit delta. Cell. Physiol. Biochem. 30, 1310–1318.
[4] Li, H. and Yang, B.B. (2012) Stress response of glioblastoma cells mediated by miR-17-5p targeting PTEN and the passenger strand miR-17-3p targeting MDM2. Oncotarget 3, 1653–1668.
[5] Venkataraman, S., Birks, D.K., Balakrishnan, I., Alimova, I., Harris, P.S., et al. (2013) MicroRNA 218 acts as a tumor suppressor by targeting multiple cancer phenotype-associated genes in medulloblastoma. J. Biol. Chem. 288, 1918–1928.
[6] Yan, Y., Luo, Y.C., Wan, H.Y., Wang, J., Zhang, P.P., et al. (2013) MicroRNA-10a is involved in the metastatic process by regulating Eph tyrosine kinase receptor A4-Mediated epithelial–mesenchymal transition and adhesion in hepatoma cells*. Hepatology 57, 667–677.
[7] Chen, L., Yang, Q., Kong, W.Q., Liu, T., Liu, M., et al. (2012) MicroRNA-181b targets cAMP responsive element binding protein 1 in gastric adenocarcinomas. IUBMB Life 64, 628–635.
[8] Ferlay, J., Shin, H.R., Bray, F., Forman, D., Mathers, C., et al. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int. J. Cancer 127, 2893–2917.
[9] Cai, N., Wang, Y.D. and Zheng, P.S. (2013) The microRNA-302-367 cluster suppresses the proliferation of cervical carcinoma cells through the novel target AKT1. RNA 19, 85–95.
[10] Xu, J., Li, Y., Wang, F., Wang, X., Cheng, B., et al. (2013) Suppressed miR-424 expression via upregulation of target gene Chk1 contributes to the progression of cervical cancer. Oncogene 32, 976–987.
[11] Peng, R.Q., Wan, H.Y., Li, H.F., Liu, M., Li, X., et al. (2012) MicroRNA-214 suppresses growth and invasiveness of cervical cancer cells by targeting UDP-*N*-acetyl-alpha-D-galactosamine:polypeptide *N*-acetylgalactosaminyltransferase 7. J. Biol. Chem. 287, 14301–14309.
[12] Yang, Z., Chen, S., Luan, X., Li, Y., Liu, M., et al. (2009) MicroRNA-214 is aberrantly expressed in cervical cancers and inhibits the growth of HeLa cells. IUBMB Life 61, 1075–1082.
[13] Xie, H., Zhao, Y., Caramuta, S., Larsson, C. and Lui, W.O. (2012) MiR-205 expression promotes cell proliferation and migration of human cervical cancer cells. PLoS One 7, e46990.
[14] Yao, Q., Xu, H., Zhang, Q.Q., Zhou, H. and Qu, L.H. (2009) MicroRNA-21 promotes cell proliferation and down-regulates the expression of programmed cell death 4 (PDCD4) in HeLa cervical carcinoma cells. Biochem. Biophys. Res. Commun. 388, 539–542.
[15] Xu, X.M., Wang, X.B., Chen, M.M., Liu, T., Li, Y.X., et al. (2012) MicroRNA-19a and -19b regulate cervical carcinoma cell proliferation and invasion by targeting CUL5. Cancer Lett. 322, 148–158.
[16] Zhang, N., Li, X., Wu, C.W., Dong, Y., Cai, M., et al. (2012) MicroRNA-7 is a novel inhibitor of YY1 contributing to colorectal tumorigenesis. Oncogene.
[17] Giles, K.M., Brown, R.A., Epis, M.R., Kalinowski, F.C. and Leedman, P.J. (2013) MiRNA-7-5p inhibits melanoma cell migration and invasion. Biochem. Biophys. Res. Commun. 430, 706–710.
[18] Karsy, M., Arslan, E. and Moy, F. (2012) Current progress on understanding MicroRNAs in glioblastoma multiforme. Genes Cancer 3, 3–15.
[19] Jiang, C., Yi, X.P., Shen, H. and Li, Y.X. (2012) Targeting X-linked inhibitor of apoptosis protein inhibits pancreatic cancer cell growth through p-Akt depletion. World J. Gastroenterol. 18, 2956–2965.

[20] Danson, S., Dean, E., Dive, C. and Ranson, M. (2007) IAPs as a target for anticancer therapy. Curr. Cancer Drug Targets 7, 785–794.
[21] Hunter, A.M., LaCasse, E.C. and Korneluk, R.G. (2007) The inhibitors of apoptosis (IAPs) as cancer targets. Apoptosis 12, 1543–1568.
[22] Lopes, R.B., Gangeswaran, R., McNeish, I.A., Wang, Y. and Lemoine, N.R. (2007) Expression of the IAP protein family is dysregulated in pancreatic cancer cells and is important for resistance to chemotherapy. Int. J. Cancer 120, 2344–2352.
[23] Bartel, D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116, 281–297.
[24] Lagos-Quintana, M., Rauhut, R., Lendeckel, W. and Tuschl, T. (2001) Identification of novel genes coding for small expressed RNAs. Science 294, 853–858.
[25] Kang, H.W., Wang, F., Wei, Q., Zhao, Y.F., Liu, M., et al. (2012) MiR-20a promotes migration and invasion by regulating TNKS2 in human cervical cancer cells. FEBS Lett. 586, 897–904.
[26] Zhou, J., Tian, Y., Li, J., Lu, B., Sun, M., et al. (2013) MiR-206 is down-regulated in breast cancer and inhibits cell proliferation through the up-regulation of CyclinD2. Biochem. Biophys. Res. Commun..
[27] Phillipps, H.R. and Hurst, P.R. (2012) XIAP: a potential determinant of ovarian follicular fate. Reproduction 144, 165–176.
[28] Burstein, D.E., Idrees, M.T., Li, G., Wu, M. and Kalir, T. (2008) Immunohistochemical detection of the X-linked inhibitor of apoptosis protein (XIAP) in cervical squamous intraepithelial neoplasia and squamous carcinoma. Ann. Diagn. Pathol. 12, 85–89.
[29] Cho, E.A., Oh, J.M., Kim, S.Y., Kim, Y. and Juhnn, Y.S. (2011) Heterotrimeric stimulatory GTP-binding proteins inhibit cisplatin-induced apoptosis by increasing X-linked inhibitor of apoptosis protein expression in cervical cancer cells. Cancer Sci. 102, 837–844.