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# Down Regulation of miR200c Promotes Radiation-Induced Thymic Lymphoma by Targeting BMI1

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## ABSTRACT

The miR-200c has recently been implicated in the epithelial to mesenchymal transition (EMT) process by directly target the EMT related transcriptional factors ZEB1 and ZEB2. The expression of this miRNA is inversely correlated with tumorgenecity and invasiveness in several human cancers. However, little is known about the expression and targets of the miR-200c in radiation carcinogenesis. Here in this study, using a split radiation induced thymic lymphoma (RITL) model in BALB/c mice, we found that miR-200c is down-regulated in RITL samples. Cell death and apoptosis in lymphoma cells was induced by miR-200c mimic while decreased by miR-200c inhibitor. Computational analysis found a putative target site of miR-200c in the 3′UTR of one of the polycomb group (PcG) protein BMI1 mRNA, which was verified by a luciferase reporter assay. Forced over-expression of miR-200c decreased the level of BMI1 protein and moreover, over-expression of BMI1 rescued the biological effects of miR-200c, indicating BMI1 is a direct mediator of miR-200c functions. Furthermore, the BMI1 expression level was up-regulated and inversely correlated with miR-200c in RITL samples. Finally, our data also indicates that Adenovirus over-expression of pre-miR-200c reduced tumorgenesis in vivo. Taken together, we conclude that down-regulated expression of miR-200c and up-regulation of its direct target BMI1 in radiation-induced thymic lymphoma, which may indicate a novel therapeutic method for RITL through induction of miR-200c or inhibition of BMI1. J. Cell. Biochem. 115: 1033–1042, 2014. 

**KEY WORDS:** RADIATION CARCINOGENESIS; RADIATION INDUCED THYMIC LYMPHOMA (RITL); miR-200C; BMI-1; IN VIVO TUMOR THERAPY

Firstly described by Kaplan et al. in 1953, mouse thymic lymphomas have become one of the classical models for radiation induced carcinogenesis [Kaplan, 1960; Kaplan, 1964; Finn et al., 1979; Boniver et al., 1980; Herranz et al., 2006]. Ionizing radiation (IR) caused DNA double strand breaks (DSB), which lead large mutations, such as a constitutional deletion or translocation [Kavanagh et al., 2013]. It was reported that multiple genes expressions were altered including oncogenes and tumor suppressing ones after irradiation, like K-ras, p53, ATM, mismatch repair genes, DNA-PKcs and p21, which play critical roles in the processes of radiation induced thymic lymphomas (RITL) [Fu et al., 2006; Liu et al., 2011a,b].

MicroRNAs (miRNAs, miR-s) are a class of endogenous small, non-coding RNAs with a length of 22–23 nt RNAs, and could regulate expression of hundreds of target mRNAs simultaneously in a post-transcriptional manner, thus modulating a variety of cell functions including cell proliferation, differentiation, death, tumor development and also radiosensitivity [Hao et al., 2011a,b; Liu et al., 2011a,b, d; Lin et al., 2013]. It was also reported that miRNAs might function as tumor suppressor genes and oncogenes [D′Amato et al., 2012; Dell′aversana and Altucci, 2012]. For example, dysregulation of the miR-17-92 cluster can induce B cell lymphoma while the expressing level of let-7 is associated with tumor progression and poor prognosis of lung cancer patients [Wang and Lee, 2009; John-Aryankalayil et al., 2012; Shu et al., 2012; Tian et al., 2012]. We previously reported that miR21 plays important roles in RITL by inhibiting the tumor suppressor gene βig-h3 [Liu et al., 2011b].

Jianguo Cui, Ying Cheng, and Pei Zhang contributed equally to this work.
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The miR-200c has recently been implicated in the epithelial to mesenchymal transition (EMT) process [Gregory et al., 2008; Wellner et al., 2009; Chang et al., 2011; Lin et al., 2013]. The expression of this miR is inversely correlated with tumorigenicity and invasiveness in several human cancers [Gregory et al., 2008; Wellner et al., 2009; Chang et al., 2011]. The role of miR-200c in cancer progression is based at least in part on their capacity to target the EMT factors ZEB1 and ZEB2, two transcription factors, which in turn repress expression of E-cadherin [Gregory et al., 2008; Wellner et al., 2009]. MiR-200c can also sensitize breast cancer cells to CD95 mediated cell death by directly targeting FAP-1 [Schickel et al., 2010]. It may also participate in cancer stem cell regulation, and loss of miR-200c increases the repression of E-cadherin gene by targeting Suz12, thus increase the tumor formation [Feng et al., 2012]. miR-200c can also be activated by p53, which plays a critical role in radiation induced injuries [Chang et al., 2011]. While whether it participates in RITL or the expression level of this miR in thymic lymphoma remains undetermined.

In this study, we found that down-regulated expression of miR-200c and up-regulation of its′ direct target BMI1 in radiation-induced thymic lymphoma, miR-200c repressed tumorgenecity through targeting BMI1, and over-expression of BMI1 partially rescued the effects of miR-200c.

## MATERIALS AND METHODS

### MICE AND TREATMENT

Split Radiation ($4 \times 1.75$ Gy) -induced thymic lymphoma model was as described in our previous work [Fu et al., 2006; Liu et al., 2011a,b]. Briefly, BALB/c mice were obtained from the Second Military Medical University, China in accordance with the Guide for Care and Use of Laboratory Animals published by the US NIH (publication No. 96-01). The animals were housed in individual cages in a temperature-controlled room with a 12 h light/dark cycle and food and water were provided ad libitum and were used at 6–8 weeks of age. Split Radiation-induced ($4 \times 1.75$ Gy) thymic lymphoma model was as described in our previous work and a $^{6°}$Co irradiator was introduced for total body irradiation (TBI) irradiation also as described in our previous work [Fu et al., 2006; Liu et al., 2011a,b,c; Cui et al., 2013]. Totally 200 mice were used in this study. The dose rate was 0.875 Gy/min.

### CELL LINES, CELL CULTURE AND TRANSFECTION

NIH3T3 cells, B16 cells, and EL4 cells were cultured and transfected as described previously [Lin et al., 2013; Hao et al., 2011a,b; Liu et al., 2011a,b,d]. Briefly, cells were maintained in Dulbecco′s modified Eagle′s medium supplemented with 10% fetal bovine serum (PAA, Austria). NIH3T3 cells were seeded in 24 well plates at a $6 \times 10^4$ cells per well and transfected with synthetic miR-s (miR-200c mimics or its inhibitor, Genema, Shanghai) at a concentration of 50 nM using Lipofectamine 2000 (Invitrogen, Canada) transfection reagent according to the manufacturer′s instructions. EL4 cells were transfected with Amaxa electroporation apparatus (Amaxa Biosystems, Koln, Germany) using manufacturer′s protocols as described previously. Besides, EL4 cells were also infected with Adenovirus over-expressing pre-miR-200c vector to up-regulate the miR-200c expression [Su et al., 2008; Wang et al., 2008; Fang et al., 2010]. Cells were used for further experiment 48 h later. For co-transfection of miR200c and BMI, Adenovirus over-expressing pre-miR-200c vector infected EL4 cells were transfected with a pcDNA3.1-BMI vector (3′UTR depleted) by using an Amaxa electroporation apparatus (Amaxa Biosystems, Koln, Germany).

### RNA EXTRACTION AND REAL TIME q-PCR

RNA from thymic tissues of differently groups were homogenized in Trizol (Invitrogen) and isolated according to the manufacturer′s instructions. Reverse transcription and Real-time PCR was subsequently performed in triplicate using the miScript RT Kit and miScript PCR system (Qiagen) as described in previous studies, while GAPDH was used as an internal control [Hao et al., 2011a; Liu et al., 2011b]. The primers used in this study were provided by the Qiagen Company. Also, the miR-200c level was detected by using the Roche technology methods [Lin et al., 2013].

### PROLIFERATION ASSAY

Cells, seeded into 96-well-plate at 4,000 cells/well were transfected with miR-200c/inhibitor/negative control and other vectors. Cell number was counted by three different methods: Directly Flowcytometry counting, MTT assay and Cell counting Kit 8 (CCK8) analysis to detect the viable, proliferating cells at 24, 48, and 72 h after transfection as described in our previous work [Hao et al., 2011a,b; Liu et al., 2011b,d; Cui et al., 2013; Lin et al., 2013].

### APOPTOSIS ASSAY

After different treatments, cells were labeled with annexin V-FITC and propidium iodide (PI) following the manufacturer′s instructions as described previously [Cui et al., 2013; Lin et al., 2013; Liu et al., 2011b,d]. Samples were determined by FACS and the results were analyzed using CellQuest software (Becton Dickinson, San Jose, CA) as described previously [Lin et al., 2013; Liu et al., 2011b,d].

### ADENOVIRUS OVER-EXPRESSING pre-miR-200c

Adenovirus over-expressing pre-miR-200c and control vector were constructed by Laboratory of Viral and Gene Therapy, Eastern Hepatobiliary Surgical Hospital, Second Military Medical University, Shanghai, China [Su et al., 2008; Wang et al., 2008; Fang et al., 2010]. Briefly, pre-miR-200c were firsty amplified the by PCR using primers: CCGGAATTCAGGGCTCACCAGGAAGTGTC (LEFT) and ACGCGTCGACGGCCCATTGTGTCCCTTAGT (RIGHT) and the murine genome cDNA were as a template. The sequence of mmu_pre-miR-200c were TGACCCCCCGGTTTTCCCCTGGAAATTCCATATTGGCACGCATTCTATTGGCTGAGCTGCGTTCTACGTGGGTATAAGAGGCGCGACCAGCGTCGGTACCGTCGCAGTCTTCGGTCTGACCACCGTAGAACGCAGATCGAATTACTAGTCAGGAATTCGCCACAGACAGGAAACACAGTTGTGAGGAATTACAACAGCCTCCCGGCCAGAGCTGGAGAGGTGGAGCCCAGGTCCCCTCTAACACCCCTTCTCCTGGCCAGGTTGGAGTCCCGCCACAGGCCACCAGAGCGGAGCAGCGCAGCGCCCTGTCTCCCAGCCTGAGGTGCAGTGCTGCATCTCTGGTCAGTTGGGAGTCTGAGATGAAGCACTGTAGCTCAGGAAGAGAGAAGTTGTTCTGCAGCCATCAGCCTGGAAGTGGTAAGTGCTGGGGGGTTGTGGGGGGCCATAACAGGAAGGACAGAGTGTTTCCAGACTCCATACTATCAGCCACTTGTGATGCTGGGGAAGTTCCTCTACACAAGTTCCCCTGGTGCCACGATCTGCTTCACGAGTCTGGTCGACTTCGAGCAACTTGTTTATTGCAGCTTATAAT

GGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGC
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TATCTTATCATGTCTGGATCGTCTAGCATCGAAGATCCAATAACTTC
GTATAGCATACATTATACGAAGTTATAAGTAGCTTGGCGTAATCAT
GGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTTCA
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CTAATGAGTGAGCTAACTCACATTAATTGCGTTGCCGCTCCACTGC
CCGCTTTCCAGTCGGGAAACCTTGTCGTGCCAGCTGCATTAAATGA
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TCGCTTCTTCGCTCCACTGACTCGCTGCGCTCGGTCGTTCGCTGCGCG
AGCGTATCAGCTCACTCAAGGCGTAATACGGTTAATCCCCAGATCA
GGATAACGCAGAAGAACTGGTGAGCCATAGCAGCAAAGCTAGGA
TCGTAAAGGCCGGATGCTGGCTTTCCCATGTCGCCCTTGACGACTCC
AATCGAGCCCTTAAGTCTCAAGAGGTTGAGTGCTAAC; All PCR products were verified by DNA sequencing and by Blasting with the NCBI Database. The sequence was inserted into a pAdTrack plasmid, named pAdTrack-CMV-miR-200c. pAdTrack-CMV-miR-200c and pAdEasy-1 were then homologously recombinated in the bacteria BJ5183. The newly recombinated plasmid, pAd-miR-200c, was tested by sequencing. Ad-miR-200c was propagated in HEK293 cells; viruses were collected from these cells, and stored at −70°C.

### IN VIVO TUMORGENECITY ASSAY

EL4 cells & B16 cells transfected with ad-virus or negative control were injected into 6-week-old male BALB/c nu/nu mice according to previous studies [Wei et al., 2008, 2009; Hou et al., 2011; Li et al., 2011; Jing et al., 2012]. Four weeks later, mice were killed for the detection of incidence of lymphomagenesis.

### miRNA-TARGET PREDICTION AND LUCIFERASE ASSAYS

The miRNA database TargetScan version 5.1 (http://www.targetscan.org/index.html) was used to identify potential targets for miR-200c and to compare the miR-200 seed sequence with the 3′UTRs of BMI1 from different species. Luciferase assays was carried out in NIH3T3 cells as described previously [Hao et al., 2011b; Hou et al., 2011; Li et al., 2011; Liu et al., 2011b; Lin et al., 2013]. Cells were transfected with appropriate plasmids in 24-well plates, and 48 h later, the cells were harvested and lysised for luciferase assay, which were performed by using a luciferase assay kit (E1910, Promega) according to the manufacturer's instructions. TK renilla was used for normalization in Dual Luciferase assays.

### PLASMIDS

The luciferase-3′UTR reporter constructs were generated by introducing the wild type and mutated 3′-UTR into pGL3 promoter vector (Promega) by the *Xba*1 site (Generay, shanghai, China) in a method described in our published papers [Hao et al., 2011a,b; Liu et al., 2011b; Lin et al., 2013]. All PCR products were verified by DNA sequencing.

### WESTERN BLOT ASSAY

Western blot analysis was performed as described before [Hao et al., 2011b; Liu et al., 2011b; Lin et al., 2013]. After determination for the concentration of protein using a BCA kit, samples were separated by a 12% SDS–PAGE and transferred to Protran nitrocellulose membranes (Schleicher and Schuell). Specific antibody for murine BMI1 (PTG, Chicago, 1:1,000) was used to detect the protein level of BMI1, while donkey anti-goat IgG-horse radish peroxidase conjugated antibodies as secondary antibodies (Cell Signaling, 1:10,000). And chemiluminescent was measured by Supersignal West Femto Maximum Sensitivity Substrate (Pierce) while the anti-beta-actin from Santa Cruz was used for normalization.

### STATISTICAL ANALYSIS

Student's *t*-test was used for comparisons between experimental groups and relevant controls. $P < 0.05$ was considered to be significantly different. The inverse correlation of BMI1 protein and miR-200c expression levels was performed by Spearman correlation analysis [Liu et al., 2011b].

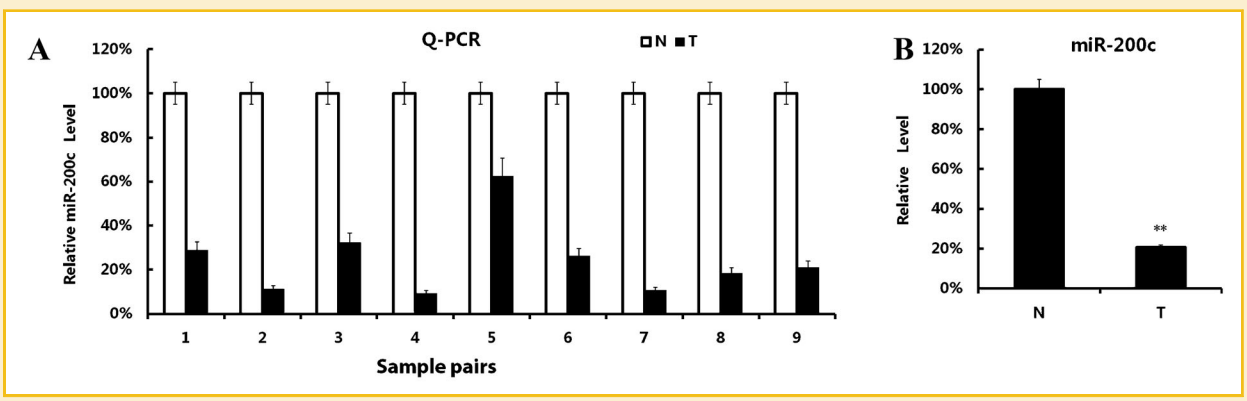


**Fig. 1.** MiR-200c was Down-regulated in radiation induced lymphoma tissues. A: The relative expression of miR-200c in nine pairs of radiation induced lymphoma tissue samples (T) and normal control thymus tissue samples (N) were detected using real-time PCR. The relative expression level of miR-200c in normal control thymus tissue samples were normalized as 1 (100%). B: A bar graph of miR-200c expression in all radiation induced lymphoma tissue samples (T) and normal control thymus tissue (N). $^{**}P < 0.01$ versus the control group.

## RESULTS

### miR-200c WAS DOWN-REGULATED IN RITL TISSUES

Here we determined the level of miR-200c in ten pairs RITL samples using a real time q-PCR assay. miR-200c was significantly down-regulated in these tumors compared to normal thymus tissues (Fig. 1A and B), indicating that miR-200c might have a role in RITL.

### miR-200c AFFECTS CELLS PROLIFERATION AND APOPTOSIS

Cell proliferation and apoptosis were determined 24 h later after transfected with miR-200c mimics/inhibitor/negative control in

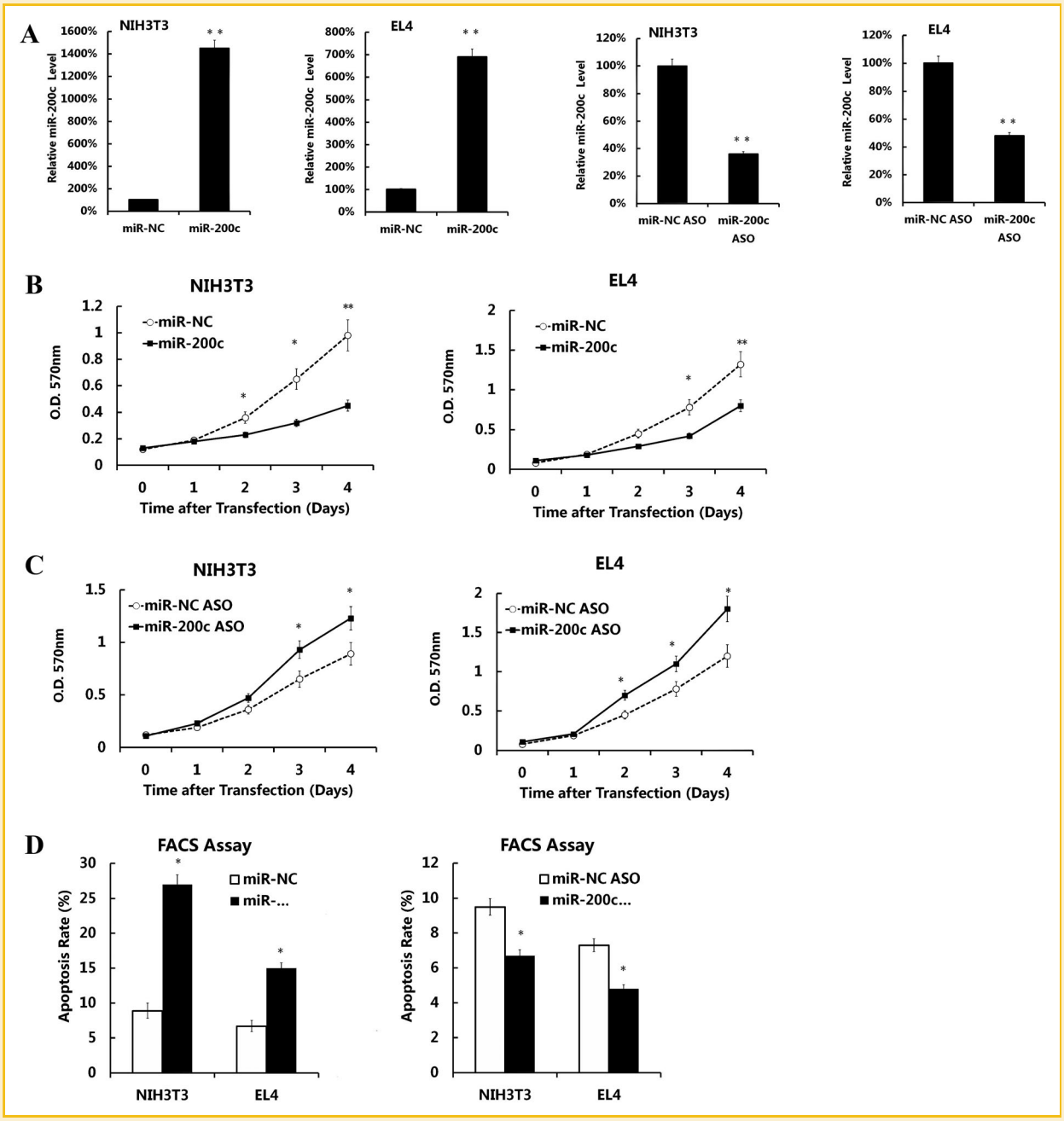


Fig. 2. MiR-200c suppressed the survival of lymphoma cells. A: Bar graphs of the relative expression of miR-200c in NIH3T3 and EL4 cells transfected with miR-200c over-expression mimics (miR-200c), miR-200c specific inhibitor antisense oligo (miR-200c ASO), or nonspecific control miRNA mimics (miR-NC). $^{**}P < 0.01$ versus the control group. B: Line graphs of cell proliferation assay determined by MTT cell counting analysis different time after transfection of the miR-200c mimics or negative control miRNA mimics (miRN-NC) in NIH3T3 cells (left) and EL4 cells (right). $^{*}P < 0.05$, $^{**}P < 0.01$ versus the control group. C: Line graphs cell proliferation as assessed by the MTT cell counting analysis different time after transfection of the miR-200c specific inhibitor antisense oligo (miR-200c ASO) or negative control miRNA inhibitor antisense oligos (miRN-NC ASO) in NIH3T3 cells (left) and EL4 cells (right). $^{*}P < 0.05$ versus the control group. D: MiR-200c (left) promoted apoptosis while miR-200c specific inhibitor antisense oligo (miR-200c ASO; right) reduced apoptosis in NIH3T3 and EL4 cells as analyzed using flow cytometry. $^{*}P < 0.05$ versus the control group.

NIH3T3 cells and EL4 cells. It was shown that miR-200c over-expression mimics (miR-200c) and miR-200c specific inhibitor antisense oligo (miR-200c ASO) could up-regulate and down-regulate the level of miR-200c when compare to nonspecific control miRNA mimics (miR-NC) or nonspecific control miRNA inhibitor antisense oligo (miR-NC ASO; Fig. 2A). Further, over-expression of miR-200c strongly inhibited the proliferation of both NIH3T3 cells and EL4 cells (Fig. 2B), while inhibition of miR-200c promoted cells proliferation (Fig. 2C). We also found that cell apoptosis was slightly increased by over-expression of miR-200c, while reduced after miR-200c inhibitor transfection (Fig. 2D).

### ADENOVIRUS OVER-EXPRESSING pre-miR-200c VECTOR REDUCED TUMORGENESIS IN VIVO

To evaluate the effects of miR-200c on tumorgenecity, we infected EL4 cells with adenovirus over-expressing of pre-miR-200c or negative Controls. Adenovirus over-expressing pre-miR-200c vector was found to be more effective in up-regulation of miR-200c in EL-4 cells in vitro (Fig. 3A). Then, $10^6$ infected EL4 cells were injected in the back of the NOD/SCID mice. Mouse lymphoma cells infected with control adenovirus formed six tumors out of six injections, whereas mouse lymphoma cells infected with adenovirus over-expressing of pre-miR-200c formed only one tumor out of six injections (Fig. 3B). The EL4/Ad-miR-200c cells formed smaller tumors than EL4/Ad-NC group (Fig. 3C). Also, Adenovirus over-expressing pre-miR-200c vector was found to suppress B16 cells in vivo (Fig. 3D andE). This suggests that miR-200c exhibit a strong suppressive effect on lymphomagenesis in vivo.

### miR-200c DIRECTLY TARGETING BMI1

Potential molecular targets of miR-200c were predicted by Target Scan5.1. The 3′-UTR of the polycomb group (PcG) protein BMI1 was screened for complementarity to seed sequences of miR-200c via a bioinformatics search. As shown in Figure 4A, wild type of mouse BMI1 3′UTR carries a putative miR-200c binding site while the mutated mouse BMI1 3′UTR is unable to bind miR-200c. It was shown that miR-200c significantly reduced the luciferase activity of the wild type BMI1 3′UTR with respect to the negative control. However, miR-200c has no effect on the luciferase activity of the mutated BMI1 3′UTR (Fig. 4B). These data indicated that miR-200c may target BMI1 in a 3′UTR dependent manner. It was shown that the amount of BMI1 protein was decreased after over-expression of miR-200c as detected by Western Blot assays and a FACS assay (Fig. 4C–E), suggesting that miR-200c negatively regulates endogenous BMI1 protein expression through a translational repression mechanism. Further, FACS assay showed that BMI1 protein expression was up-regulated in RITL samples, which was inversely related with the miR-200c level (Fig. 5).

### 3′UTR DEPLETED BMI1 PARTIALLY RESCUED miR-200c MEDIATED APOPTOSIS ON LYMPHOMA CELLS

To investigate whether the effects of miR-200c was mediated by BMI1, EL4 cells were co-transfected a pcDNA3.1-BMI vector (3′UTR depleted) with or without miR-200c expressing adenovirus.

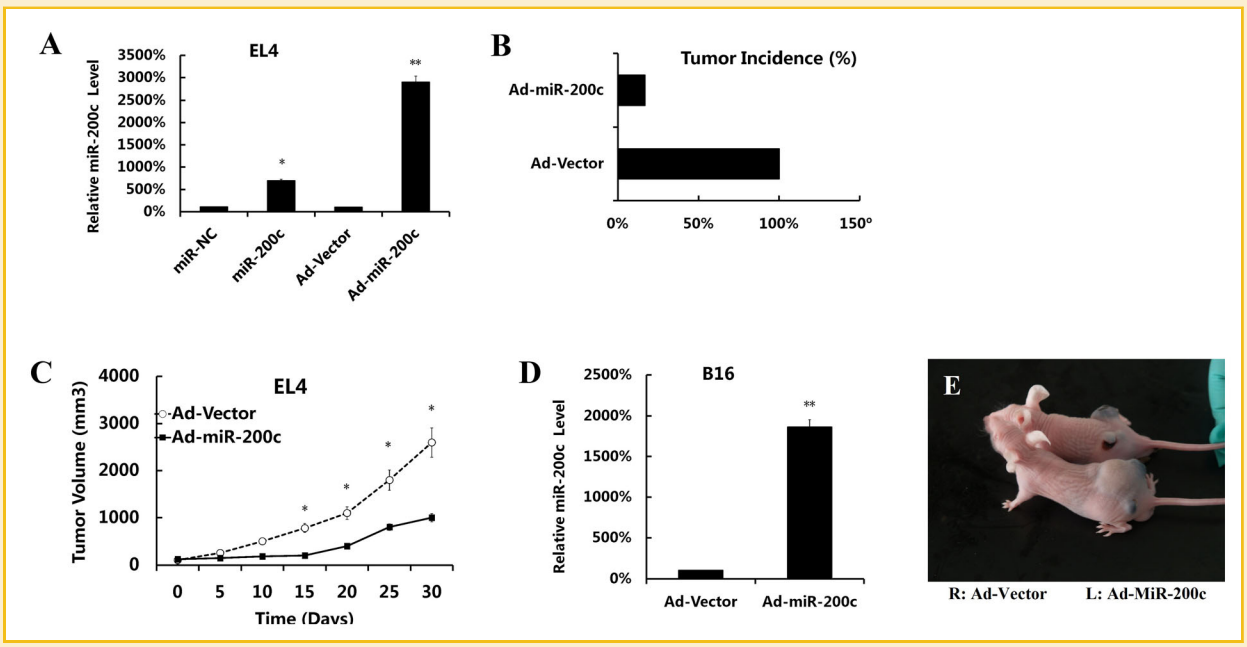


**Fig. 3.** Adenovirus over-expressing pre-miR-200c vector reduced tumorgenesis in vivo. A: Validation of miR-200c over-expression Adenovirus in EL4 cells 24 h after infection with adenovirus over-expressing pre-miR-200c vector or negative control adenovirus vector, the relative levels of miR-200c were analyzed by q-RT-PCR. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the control group. B: Single cell suspensions of EL4 cells were either infected with adenovirus over-expressing pre-miR-200c vector or negative control adenovirus vector, and 48 h after infection, lymphoma cells ($3 \times 106$) were subcutaneously injected into the backs of nude mice. The tumor formation incidence of the transplanted tumors were measured. C: A line graphs of tumor size which was measured twice weekly for 30 days. $^{*}P < 0.05$ versus the control group. D: Validation of miR-200c over-expression Adenovirus in B16 cells. $^{**}P < 0.01$ versus the control group. E: Representative images of mice bearing adenovirus over-expressing pre-miR-200c vector or negative control adenovirus vector infected B16 cells were shown.

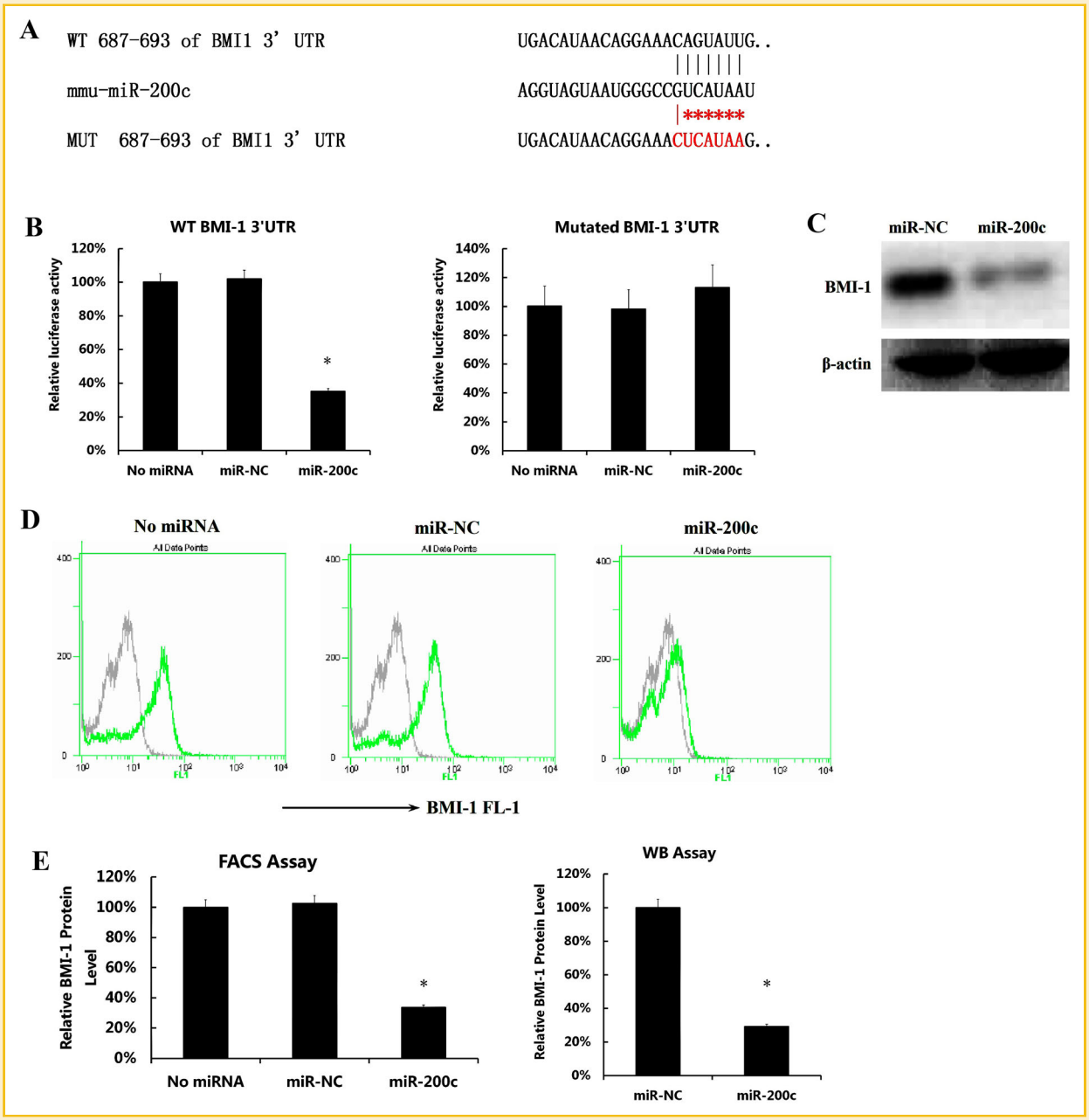


Fig. 4. miR-200c targets BMI1 in a 3′UTR dependent manner. A: The predicted miR-200c binding site on murine BMI1 mRNA 3′UTR and the mutation strategy is shown. B: A dual luciferase assay of NIH3T3 cells co-transfected with the firefly luciferase constructs containing the wild type (left) or mutated BMI1 3′-UTR (right) and miR-200c mimics or scrambled negative control (miR-NC). $^{*}P < 0.05$ versus the control group. C: miR-200c mimics inhibit endogenous BMI1 expression in EL4 cells as detected by WB assay. D: miR-200c mimics inhibit endogenous BMI1 expression in EL4 cells as detected by FACS assay. E: bar graphs of expression of BMI determined by FACS and WB. Statistical data of C & D. $^{*}P < 0.05$ versus the control group.

Consistent with our data, the survival rate was significantly decreased in the miR-200c treated groups. But in the miR-200c and BMI1 co-transfected group, the survival were increased significantly (Fig. 6), indicating BMI1 rescued the cell killing effects of miR-200c.

## DISCUSSION

Radiotherapy is now an important method for treating cancer [Corvo et al., 1997; van Zijtveld et al., 2007; Schenck et al., 2010; Liu et al., 2011d]. Radiation carcinogenesis is also one of the severest outcomes for people exposed to ionizing radiation [Liu et al., 2011b]. MiR-s has been proposed to function as tumor suppressors or oncogenes in tumor genesis and development, and they may also play critical roles in carcinogenesis [Liu et al., 2011b,d; Lin et al., 2013]. Epigenetically silence of miR-137 was an early event in colorectal carcinogenesis [Bandres et al., 2009; Balaguer et al., 2010; Chen et al., 2013]. Down-regulation of miR26a contributed to carcinogenesis in breast cancer by targeting MTDH and EZH2 [Wong and

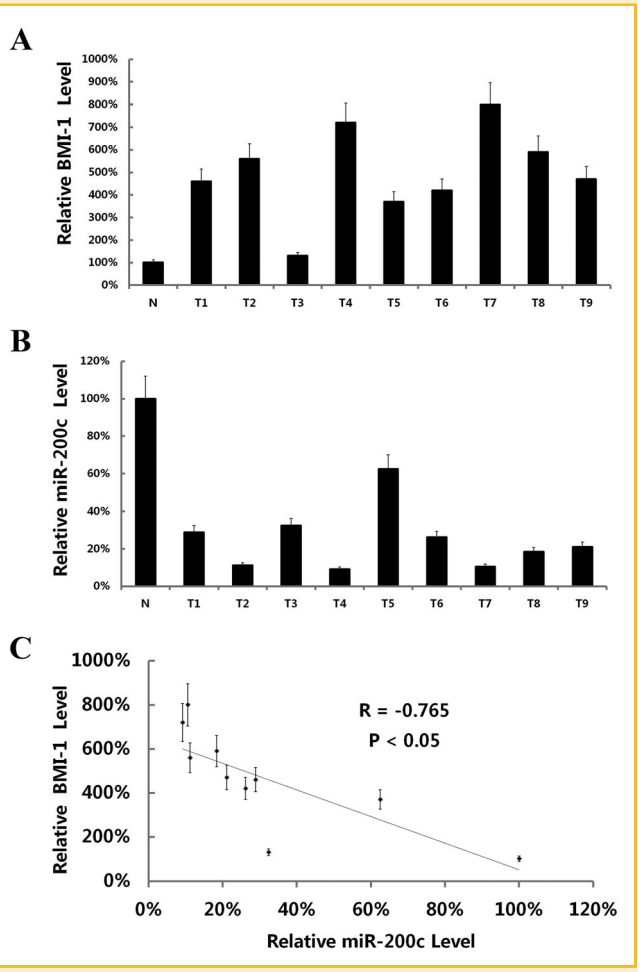


**Fig. 5.** BMI1 expression level was up-regulated and inversely correlated with miR-200c in split radiation induced lymphoma tissue samples. A: BMI1 expression level was up-regulated in split radiation induced lymphoma tissue samples: FACS assay. T, radiation induced Thymic lymphoma tissue samples (T) and N, normal control thymus tissue samples. B: Expression of miR-200c in normal thymus tissue samples and radiation induced Thymic lymphoma tissue samples. C: BMI1 protein and miR-200c expression levels are inversely correlated in radiation induced thymic lymphoma tissue samples. The inverse correlation of BMI1 protein and miR-200c expression levels was examined by Spearman correlation analysis. $P < 0.05$.

Tellam, 2008; Dang et al., 2012]. miR-1 was found to suppress thyroid carcinogenesis by targeting CCND2, CXCR4, and SDF-1 [Leone et al., 2011]. miR-18a*, miR-132, miR-146a, miR-211 and so on were also reported to function as tumor suppressor or onco-miRs in carcinogenesis [Wang and Lee, 2009]. Our department has focused on miRNA in radiation induced carcinogenesis and tumor radiosensitivity. We have found that Bigh3 and miR-21 played critical roles in radiation induced lymphoma while miR-200c could enhance radiosensitivity in breast cancer cells [Liu et al., 2011b; Lin et al., 2013].

In this study, we found that miR-200c was strongly down-regulated in RITL tissues. Consistent with our study, miR-200c was also reported to be down-regulated in many other types of cancers [Chen et al., 2012; Cittelly et al., 2012; Lin et al., 2013]. Then we investigated the effects of miR-200c on cell proliferation, apoptosis, tumorgenesis, and identified one of its targets. And our data shown that over-expression of miR-200c inhibited cells proliferation and induced cells apoptosis, while adverse effects were observed when miR-200c was knocked down. miR-200c could also suppress tumor genesis capacity in vivo. To our knowledge, this may be the first report about the expression changes of miR-200c in RITL model.

miR-200c has been shown to be a direct target of p53 transcription factor [Boominathan, 2010; Chang et al., 2011; Schubert and Brabletz, 2011; Xie et al., 2013], which was identified as a critical tumor suppressor gene which coordinated responses to stress conditions [Chang et al., 2011]. P53 could also inhibit cell proliferation through cell cycle arrest and induction of apoptosis [Boominathan, 2010; Chang et al., 2011]. In this study, we also found that over-expression of miR-200c caused similar effects, inhibiting proliferation and induction of apoptosis. P53 mutation was often found in many types of cancers [Li and Lu, 1994; Daya-Grosjean et al., 1995; Schlechte et al., 1997], and we concluded in our model, down-regulation of miR-200c might be a downstream effect of p53 mutation, because it could not transactivate miR-200c any longer. And in our in vivo study, we found that miR-200c expressing adenovirus could strongly suppress the tumorgenecity of EL4 cells, which suggested the important role of miR-200c in lymphoma treatment. It is concluded that miR-200c functions similarly like the tumor suppressor gene p53.

But how could miR-200c mediated the tumor suppressive effects? By miRNA target prediction and dual reporter gene, we confirmed oncogene BMI1 as a directly target of miR-200c. And down-regulation of miR-200c increased the BMI1 gene expression, which promoted the process of RITL. BMI1 has been reported as an oncogene by regulating cell cycle inhibitors, p16 and p19 [Lessard et al., 1999; Fan et al., 2008; Tirabosco et al., 2008; Chatoo et al., 2010; Smith et al., 2011; Allegra et al., 2013; Biehs et al., 2013; Gargiulo et al., 2013]. It was also thought to be necessary for efficient self-renewing cell divisions for many types of stem cells [Chatoo et al., 2010; Biehs et al., 2013]. And we found that over-expressing of 3′UTR depleted BMI1 could reverse apoptosis induction effects by miR-200c. These data are consistent with previous reports and only in different cell type [Kopp et al., 2012]. It was also reported that over-expression of BMI1 could cause p53 suppression, which provided evidence for the existence of some positive feedbacks [Alajez et al., 2009; Caramel et al., 2011; Calao et al., 2012].

Radiation induced carcinogenesis is a multiple steps, complex process and the underlying mechanism remains unclear [Liu et al., 2011b,d]. In this study, we demonstrated that down-regulated expression of miR-200c and up-regulation of its direct target BMI1 in radiation-induced thymic lymphoma, which may indicate a novel therapeutic method of that cancer. And our data suggests novel treatments through up-regulating the level of miR200c while down-regulating the level of BMI for the therapy of RITL.

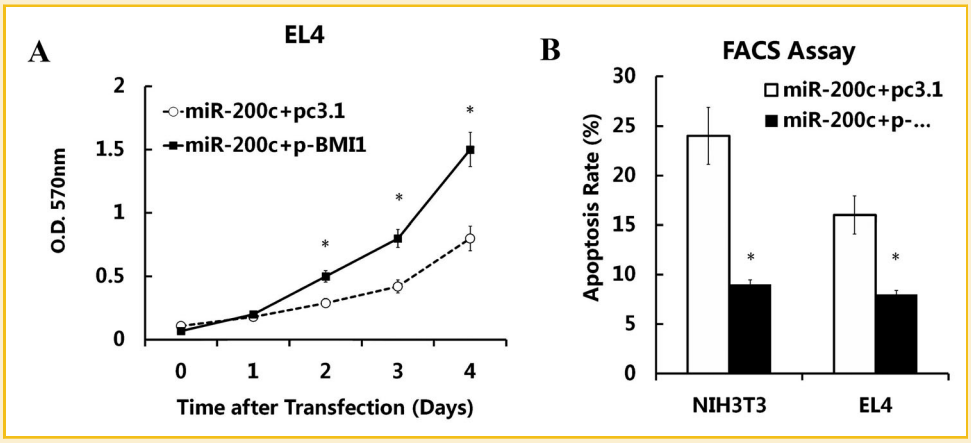


**Fig. 6.** Rescue effects of 3′UTR depleted BMI1 in cells ectopically expressing of miR-200c. (A). Over-expression of 3′UTR depleted BMI1 attenuated the anti-proliferation effect of miR-200c; Cell Number counting by FACS Assay. $^{*}P < 0.05$ versus the control group. (B) Over-expression of 3′UTR depleted BMI1 attenuated the apoptosis rate in cells with miR-200c over-expression. Ann-V/PI double staining assay and detected by FACS Assay; $^{*}P < 0.05$ versus the control group.

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