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MicroRNA-26b Enhances the Radiosensitivity of Hepatocellular Carcinoma Cells by Targeting EphA2

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Sensitizing hepatocellular carcinoma (HCC) cells to irradiation is important to achieve satisfactory therapeutic effect with low-dose radiotherapy. Erythropoietin-producing hepatocellular carcinoma A2 (EphA2) is a member of the Eph receptor family that constitutes the largest family of tyrosine kinase receptors. EphA2 overexpression is one of the poor prognostic factors in many progressive cancers. Importantly, EphA2 is a potential target of microRNA-26b (miR-26b), and miR-26b expression is down-regulated in several types of cancer. In this study, we measured the expression levels of miR-26b and EphA2 protein in seven human HCC cell lines by quantitative PCR and western blot analysis, respectively. Overall, lower miR-26b expression levels tended to be associated with higher EphA2 levels in HCC cell lines. Among the cell lines examined, 97H HCC cells expressed the lowest level of miR-26b and highest level of EphA2 protein. Thus, using 97H HCC cells, EphA2 mRNA was verified as the target of miR-26b by the luciferase reporter assay. Accordingly, a synthetic miR-26b, miR-26b mimics, was used to mimic the function of endogenous miR-26b. In 97H HCC cells transfected with miR-26b mimics or short-hairpin RNA targeting EphA2 mRNA, expression of EphA2 protein was reduced, which was associated with significantly lower proliferation rate and invasion ability and with higher apoptosis rate in response to low-dose irradiation, compared to control cells. In contrast, 97H HCC cells over-expressing EphA2 showed higher proliferation rate and invasion ability and lower apoptosis rate upon irradiation. These data suggest that miR-26b enhances the radiosensitivity of 97H HCC cells by targeting EphA2 protein.

Keywords: EphA2; 97H HCC cells; hepatocellular carcinoma; miR-26; radiosensitivity
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Introduction

Hepatocellular carcinoma (HCC) currently ranks as the sixth most common and third most deadly cancer worldwide, posing a huge threat to human life (Hung et al. 2014). HCC usually occurs in the context of a diseased cirrhotic liver with poor hepatic reserves, which makes it difficult to undertake conventional high-dose radiotherapy (RT), as high-dose irradiation inflicts serious damage not only to HCC but also to hepatic tissues (Ursino et al. 2012; Hung et al. 2014). Low-dose RT, a recent advance in the field of RT, is characterized by little collateral damage to neighboring organs and tissues, but it needs that cancer cells (e.g., HCC) be sensitized to low-dose irradiation in order to achieve satisfactory therapeutic effect. To date, the precise molecular mechanism involved in modulating the radiosensitivity of HCC cells is poorly defined, which hinders extensive clinical application of low-dose RT in HCC.

MicroRNA (miRNA), a class of small endogenous non-coding single-stranded RNA, drew great attention after many of them was found to be aberrantly expressed in diverse kinds of cancers (Wang et al. 2009; Ostenfeld et al. 2010; Salim et al. 2012). Ongoing research has identified miRNAs that tightly correlate with multiple hallmarks of cancer cells, such as radiosensitivity, proliferation, migration, and invasion. With regard to the radiosensitivity of cancer cells, microRNA-181 (miR-181), for example, sensitizes human gliomas to irradiation by targeting Bcl-2 (Chen et al. 2010). miR-221 or miR-222 increases the susceptibility of gastric carcinoma cells to irradiation by targeting PTEN (Chun-Zhi et al. 2010). miR-26b has been observed to be down-regulated in many cancers and is commonly associated with cancer development and worst outcome after cancer therapy (Shen et al. 2014). However, to date, the role of miR-26b in the radiosensitivity of cancer cells, especially HCC, is barely understood.

Erythropoietin-producing hepatocellular carcinoma A2 (EphA2), acting as an important member of the Eph receptor family that constitutes the largest family of tyrosine kinase receptors in mammals, is highly expressed in many cancers such as breast cancer, prostate cancer, lung cancer, malignant glioma, and gastric cancer (Mosch et al. 2012).

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EphA2 is essential for the regulation of several processes during the embryonic morphogenesis and modulates tissue renewal and homeostasis in the adult (Callegari et al. 2015; Zhou et al. 2015). A previous study found that up-regulated EphA2 expression is associated with cancer lymphogenous metastasis and poor prognosis, suggesting that EphA2 contributes to cancer progression (Mosch et al. 2012). Likewise, EphA2 increased tumorigenesis, migration and invasion of tumor cells, angiogenesis, and metastasis (Annamalai et al. 2009). Moreover, EphA2 provides a protective mechanism by which tumors can attenuate irradiation-induced antivascular effect, so that the tumors can easily migrate and escape radiation (Fokas et al. 2010). Previous literature represents EphA2 as an important target of miR-26b in gliomas (Wu et al. 2011). We, therefore, speculated that down-regulated miR-26b expression in many cancers is an important reason for the up-regulation of EphA2 expression. If true, over-expressing miR-26b to reduce EphA2 expression is a feasible strategy to inhibit EphA2-mediated irradiation-protective effect and to sensitize cancer cells to irradiation.

In the present study, the expression of miR-26b and EphA2 protein in multiple HCC cell lines were evaluated by qPCR and western blotting, respectively, to gather basic understanding of the expression profiles of miR-26b and EphA2 in HCC cells and their potential interrelationship. Dual luciferase reporter assay was performed to validate EphA2 as an important target of miR-26b in HCC cells. Subsequently, 97H HCC cells were separately transfected with miR-26b mimics, EphA2-small hairpin RNA (EphA2-shRNA), and EphA2 expression vector to investigate the changes in cell response to low-dose irradiation in terms of proliferation rate, apoptosis rate, and invasion ability. This study aimed to determine whether the radiosensitivity of HCC cells can be enhanced by miR-26b by targeting EphA2, which is further expected to provide a theoretical foundation for the modulation of the radiosensitivity of HCC cells during low-dose RT.

Materials and Methods

Cell culture

Human HCC cell lines, including HepG2, SMMC7721, Huh7, Bel-7402, GGY7703, 97L, and 97H, as well as the hepatic cell line L02 were purchased from the American Type Culture Collection (Manassas, VA, USA) and grown in Dulbecco's Minimal Essential Medium (Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, Utah, USA) and 1% penicillin/streptomycin at 37°C in a humidified incubator containing a mixture of 95% air and 5% CO_2.

Quantitative polymerase chain reaction (qPCR)

Total RNA from the cells was obtained using Trizol reagent (Invitrogen Life Technologies). Complementary DNA (cDNA) was synthesized using a First-Strand cDNA Synthesis kit (Fermentas, Vilnius, Lithuania) and using 1 μg of RNA template. qPCR was performed in a 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using a SYBR® Green PCR kit (Applied Biosystems). The primers of miR-26b and the housekeeping gene (U6) were as follows: miR-26b forward primer 5′-GGGACCCAG TTCAAGTAATTCAGG-3′ and reverse primer 5′-TTTGGCACTAGC ACATT-3′; and U6 forward primer 5′-CTCGCTTCGGCAGCACA-3′ and reverse primer 5′-AACGCTTCACGAATTTGCGT-3′. The relative expression level of miR-26b was calculated using comparative computerized tomography methods, with U6 as an internal control.

Western blot analysis

Cells were solubilized with the lysis buffer (1% Non-ionic detergent-40, 0.1% Sodium dodecyl sulfate, 50 mM dithiothreitol, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 1 mM phenylmethanesulfonyl fluoride) and the solution was boiled for 5-10 min, and an equal amount of protein from different groups was separated by SDS-PAGE. After electrophoresis, the proteins were transferred onto a nitrocellulose membrane. The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline and then incubated with primary antibodies directed against EphA2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 1 h at room temperature. The blots were developed using peroxidase-conjugated anti-mouse IgG secondary antibody (Santa Cruz Biotechnology, Inc.), and the proteins were visualized by enhanced chemiluminescence (Amersham Bio-sciences, NJ, USA). β-actin was used as a loading control.

Luciferase reporter assay

The predicted miR-26b-binding site (MRE, miRNA response element) in the EphA2 3′-untranslated region (UTR) was subcloned into pmirGLO (Promega, WI, USA) to construct a miR-26b MRE luciferase reporter. This reporter was introduced into 97H cells by using Lipofectamine 2000 (Invitrogen Life Technologies), according to the manufacturer's recommendation. The reporter vector without the miR-26b MRE in the EphA2 3′UTR (empty reporter vector) and the reporter vector inserted with the mutated miR-26b MRE in the EphA2 3′UTR (mutated reporter vector) were used as two control. The luciferase activity was measured at 48 h after transfection by using GloMax 20/20n luminous detector (Promega).

Transfection

To knockdown or over-express EphA2, the cells were grown in complete medium for 24 h and then transfected with short hairpin RNA targeting EphA2 mRNA, EphA2-shRNA (GenePharma, Co., Ltd, Shanghai, China), or EphA2 expression vector (pEGFP-C1; Invitrogen Life Technologies), by using Lipofectamine™ 2000 (Invitrogen Life Technologies), following the procedure recommended by the manufacturer. Importantly, the EphA2 expression vector lacks the miR-26b MRE, thereby ensuring the expression of EphA2 in cells expressing miR-26b. Thus, the construct was termed the EphA2 over-expression vector in the present study. The miR-26b mimics (GenePharma, Co., Ltd.) was added to the culture medium of 97H cells at a final concentration of 100 nM using Lipofectamine™ 2000.

Irradiation

The 97H cells were plated in 3.5-cm dishes and incubated in the culture medium until 70-80% confluence was attained. Cells were next cultured in the medium without FBS and exposed to irradiation at a dose of 1 Gy, a dose determined based on a preliminary experiment where the cells were subjected to 1, 2, 4, and 6 Gy. Seventy-two hours after irradiation, the rates of cell proliferation and apoptosis

were measured by CCK-8 assay and Annexin V-fluorescein isothiocyanate (V-FITC)/Propidium Iodide (PI) double-staining test.

Cell proliferation assay

The cells were seeded at a density of 1×10^5 cells per well into 96-well plates containing 100 μl/well of the culture medium for 24 h. One hour before the incubation period ended, 10 μl of CCK-8 reagent was added to each well. The optical density at 490 nm (OD_{490nm}) of each well was determined by an enzyme immunoassay analyzer.

Apoptosis rate assay

Cells were dual-stained with Alexa Fluor 488-Annexin V and propidium iodide (PI) by using an Annexin V-FITC/PI apoptosis kit (Kaiji Biological Inc., Nanjing, China), according to the manufacturer's instructions. The rate of apoptosis was analyzed using a dual laser flow cytometer (Becton Dickinson, San Jose, CA, USA) and estimated using the ModFit LT software (Verity Software House, Topsham, ME, USA).

Cell invasion assay

Transwell chamber (Corning, NY, USA) was used to test cancer cell invasion, with 8-μm membrane filter inserts coated with Matrigel (BD Biosciences, CA, USA). Briefly, the cells were trypsinized and suspended in serum-free medium. Next, 1×10^4/ml cells were added to the upper chamber, and the lower chamber was filled with the medium containing 10% FBS. After 36 h of incubation, the cells that had invaded the lower chamber were fixed with 95% ethyl alcohol for 15-20 min and stained with hematoxylin for 10 min. Then, the cells were enumerated microscopically.

Statistical analysis

The results are presented as the mean ± standard deviation (SD) of three independent experiments. One-way analysis of variance (ANOVA) with post-hoc testing was used for multiple comparisons between each group (SPSS13.0 software, IBM, NY, USA). Significant differences were established at $P < 0.05$.

Results

Growth property of cell lines

HCC cell lines as well as the hepatic cell line had different growth property. 97H and Huh7 cell lines exhibited most rapid proliferation with a doubling time of ~ 48 h (data not shown). In contrast, the proliferation of Bel-7402, SMMC7721, and HepG2 cells was relatively slower, and their doubling time was 2 ~ 3 days. The doubling time of GGY7703, 97L, and L02 cells was ≥ 4 days.

Expression levels of miR-26b and EphA2 protein in different HCC cell lines

The expression levels of miR-26b and EphA2 protein in different HCC cell lines were analyzed (Fig. 1). The expression levels of miR-26b tended to be lower in all tested HCC cell lines, compared to L02 normal liver cells (Fig. 1A), whereas EphA2 protein expression levels were higher in the HCC cell lines (Fig. 1B). Overall, lower miR-26b expression in HCC cell lines tended to be associated with higher EphA2 protein levels, suggesting the inverse relationship between miR-26b and EphA2 in HCC cells.

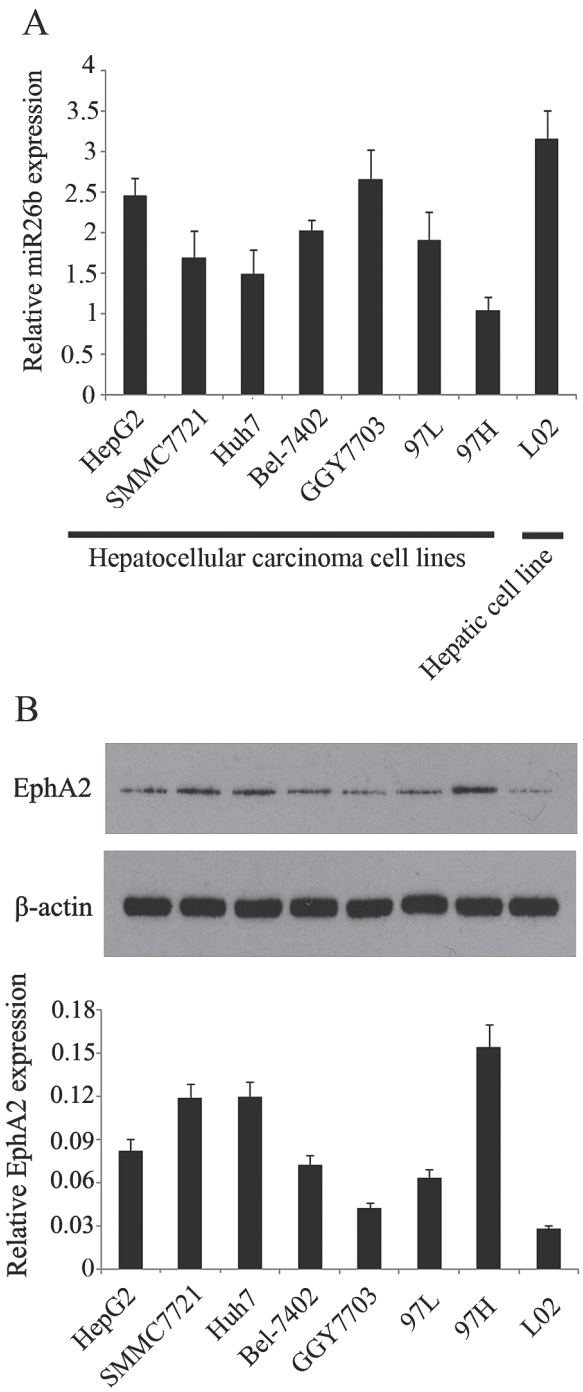


Fig. 1. Expression levels of miR-26b and EphA2 protein in different hepatocellular carcinoma (HCC) cell lines. Human HCC cell lines, including HepG2, SMMC7721, Huh7, Bel-7402, GGY7703, 97L, and 97H, and the hepatic cell line L02 were used to detect miR-26b and EphA2 protein by PCR and western blotting analysis, respectively. (A) miR-26b shows reduced expression in all HCC cell lines, compared to the hepatic cell line. (B) EphA2 protein shows increased expression in all HCC cell lines, compared to the normal hepatic cell line. Each bar represents the mean of three independent experiments.

Most obviously, 97H cells with the lowest level of miR-26b expressed highest level of EphA2 protein. Thus, 97H HCC cells were used in the following experiments.

EphA2 as a target of miR-26b in 97H HCC cells

To determine whether EphA2 is a target of miR-26b in HCC cells, we performed thel luciferase reporter assay. The predicted miR-26b-binding site sequence in the EphA2 3′UTR, cloned downstream to the luciferase reporter gene, rendered 83% decrease of luciferase activity ($P < 0.01$, Fig. 2), compared to the activity of the empty reporter vector lacking the MRE or mutated reporter vector. Thus, miR-26b may decrease the expression of EphA2 mRNA by targeting the MRE in HCC cells.

Optimum irradiation dose of 1 Gy

To obtain the optimum low dose of irradiation for use in our experiments, 97H cells were exposed to different doses of irradiation from 1 to 6 Gy. Compared to the control, irradiation with 1 Gy caused minor damage to 97H HCC cells; only marginal alterations in the proliferation rate, apoptosis rate, and invasion ability were observed after irradiation (Fig. 3A-C), but with increasing dose of irradiation, the damage inflicted by irradiation evidently increased. Irradiation with 2 Gy significantly decreased the rates of cell proliferation and invasion and increased the rate of apoptosis ($P < 0.05$). Irradiation with 1 Gy was determined as the optimum low dose of irradiation for our study, because we wanted to analyze the enhanced radiosensitivity of 97H cells.

EphA2 expression level in 97H HCC cells following different treatments

We first evaluated the effect of the transfection of non-targeting miRNA mimic, non-targeting small interfering RNA (siRNA), and empty expression vector on the EphA2 expression level in 97H HCC cells. No noticeable alteration in the EphA2 expression level was induced by the transfection, thereby excluding the possibility that the introduction of non-targeting miRNA, siRNA or the empty vector causes the change in EphA2 expression (Fig. 4A). The transfection with miR-26b mimics was used to mimic the function of endogenous miR-26b in 97H cells. Either miR-26b mimics or EphA2-shRNA transfection resulted in significant reduction of EphA2 expression ($P < 0.05$, Fig. 4B) in comparison to the non-transfected cells. In contrast, transfection with EphA2 over-expression vector caused the increase in EphA2 expression ($P < 0.05$). We further investigated the EphA2 expression level in the transfected cells upon low-dose irradiation. As shown in Fig. 4C, exposure to 1 Gy irradiation did not cause significant reduction in EphA2 expression in 97H cells. The transfection with miR-26b mimics or EphA2-shRNA, followed by the exposure to low-dose irradiation, significantly decreased EphA2 expression relative to control ($P < 0.05$ for each). Up-regulated EphA2 expression was observed in the cells transfected with EphA2 over-expression vector despite the low-dose irradiation ($P < 0.05$).

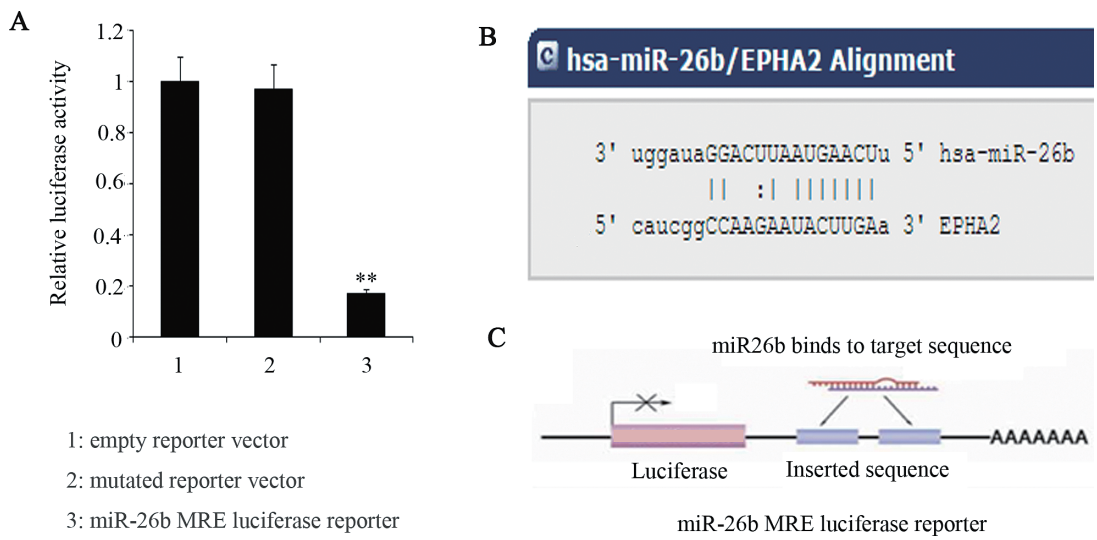


Fig. 2. EphA2 is a target of miR-26b in 97H HCC cells.
(A) Effect of the miR-26b-binding site MRE on the luciferase activity. The miR-26b MRE luciferase reporter contains the predicted miR-26b-binding site (MRE) in the EphA2 3′UTR. The reporter vector without the miR-26b MRE (empty reporter vector) and the reporter vector with the mutated miR-26b MRE (mutated reporter vector) were used as two types of negative control. Each reporter was expressed in 97H HCC cells. The luciferase activity was measured and calculated at 48 h after transfection. $^{**}P < 0.01$. (B) The predicted miR-26b-binding site sequence (MRE) in the EphA2 3′UTR (http://www.microrna.org/microrna/getMrna.do?gene=1969&utr=36217&organism=9606). (C) The schematic representation of the luciferase reporter assay. If miR-26b can bind to the inserted MRE, the luciferase expression is decreased.

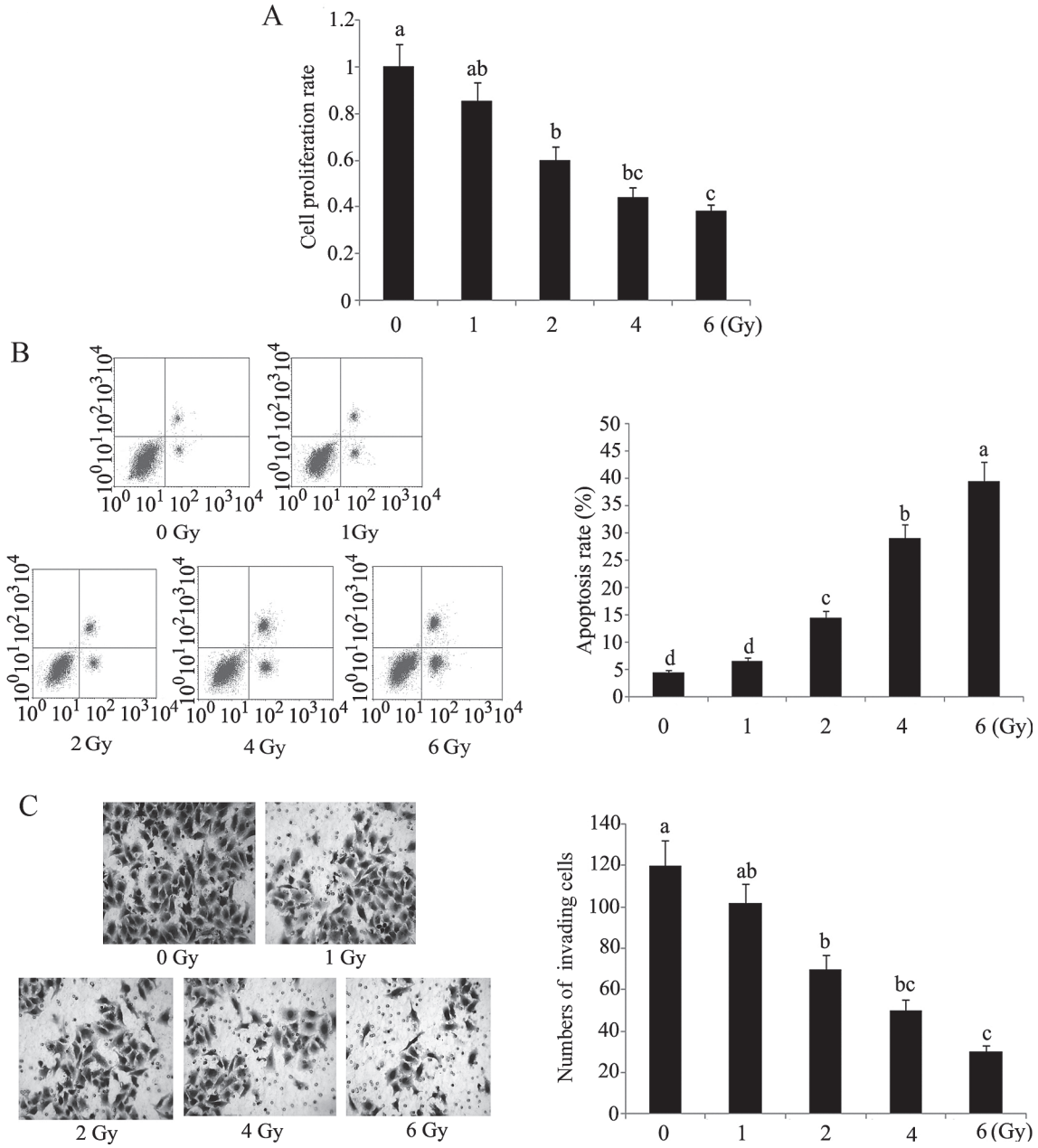


Fig. 3. Effects of different doses of irradiation on 97H HCC cells.
97H HCC cells were irradiated to different doses before testing (A) cell proliferation rate, (B) apoptosis rate, and (C) cell invasion ability using CCK-8 kits, Annexin V-fluorescein isothiocyanate/PI apoptosis kit and flow cytometer, and Transwell chambers. Each bar represents the mean of three independent experiments. Bars not sharing a common letter differ ($P < 0.05$).

Changes in the radiosensitivity of 97H HCC cells after different treatments

The radiosensitivity of 97H HCC cells after different treatments was evaluated by studying the changes in cell proliferation rate, apoptosis rate, and invasion ability. Either miR-26b mimics or EphA2-shRNA transfection caused ~ 45% decrease in the cell proliferation rate after treatment with low-dose irradiation, compared to the control (both $P < 0.05$; Fig. 5). Transfection with EphA2 over-expression vector enhanced the rate of cell proliferation, relative to the control ($P < 0.05$). Moreover, the post-irradiation apoptosis rate showed a dramatic increase after transfection with miR-26b mimics or EphA2-shRNA (both $P < 0.01$; Fig. 6). Transfection with EphA2 over-expression vector lowered the rate of apoptosis to 35% of the control cells with irradiation ($P < 0.05$). Post-irradiation cell invasion ability was remarkably attenuated in 97H cells that were transfected with miR-26b mimics or EphA2-shRNA, as determined by the decrease in the number of cells passing through the Transwell (~ 40% compared to the control; $P < 0.05$; Fig. 7, and 30% compared to the cells exposed to low-dose irradiation only; $P < 0.05$). Transfection with

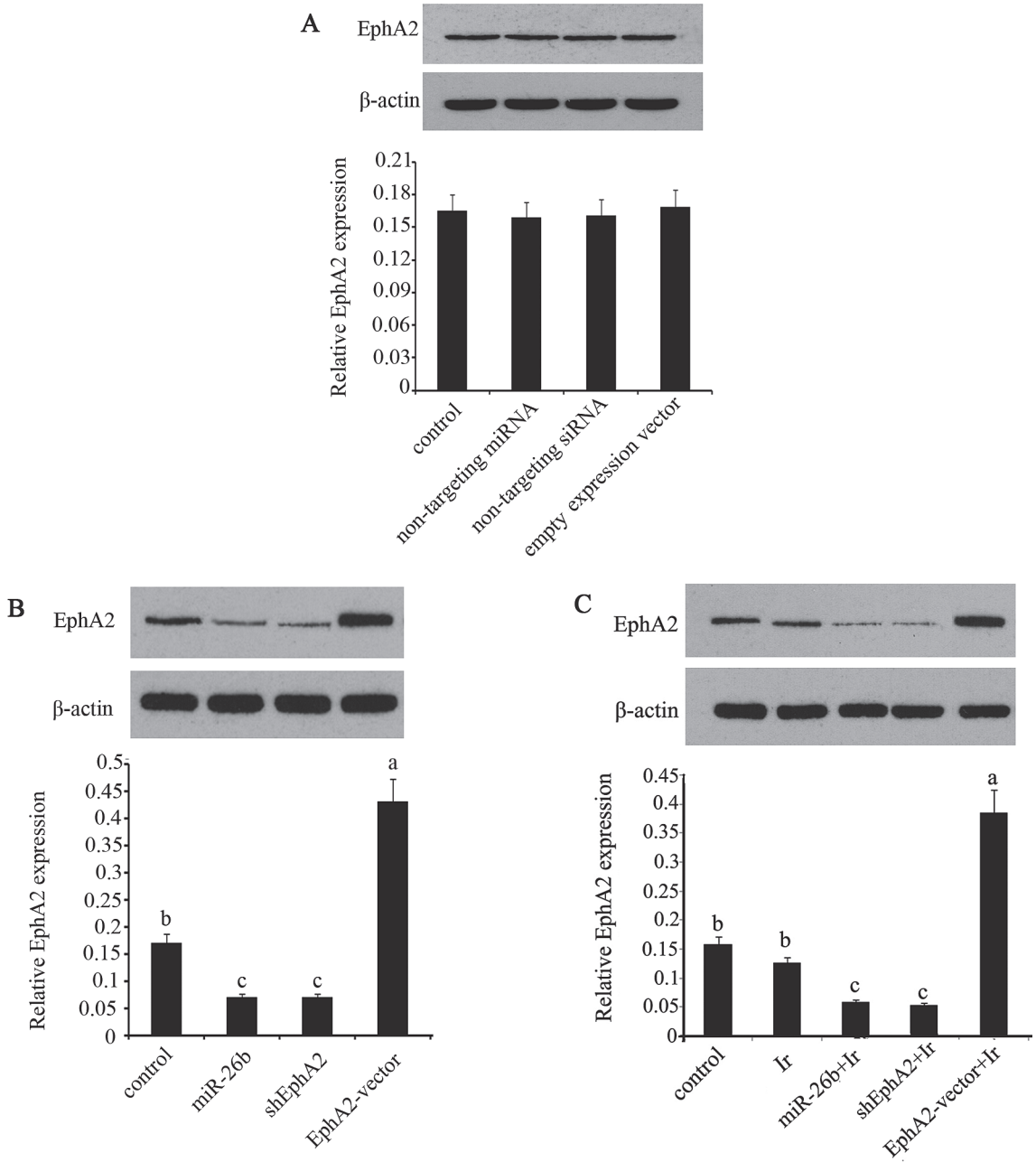


Fig. 4. EphA2 expression level in 97H HCC cells following different treatments.
(A) The EphA2 expression level in 97H HCC cells that were transfected with non-targeting miRNA mimic, non-targeting siRNA, or empty over-expression vector. (B) The EphA2 expression level in 97H HCC cells that were separately transfected with miR-26b mimics, EphA2-shRNA, and EphA2 over-expression vector. (C) 97H HCC cells were separately transfected with miR-26b mimics, EphA2-shRNA, and EphA2 over-expression vector. EphA2 expression level was assessed after these cells were exposed to 1 Gy irradiation. Each bar represents the mean of three independent experiments. Bars not sharing a common letter differ ($P < 0.05$).
Ir, irradiation; miR-26b, miR-26b mimics; EphA2-vector, EphA2 over-expression vector; shEphA2, EphA2-shRNA.

EphA2 over-expression vector conferred significant increase in the cell invasion ability even after treatment with low-dose irradiation ($P < 0.05$).

Discussion

In the present study, miR-26b showed different levels of down-regulation in multiple HCC cell lines compared to the normal hepatic cells. The level of down-regulated miR-26b is inversely correlated with the grade of HCC and its prognosis after therapy. Grade I and II liver tumors maintain relatively high levels of miR-26b expression, but grade III and IV tumors have markedly lower levels of miR-26b expression (Shen et al. 2014). In HCC, low miR-26b expression often indicated poor survival (Ji et al. 2009).

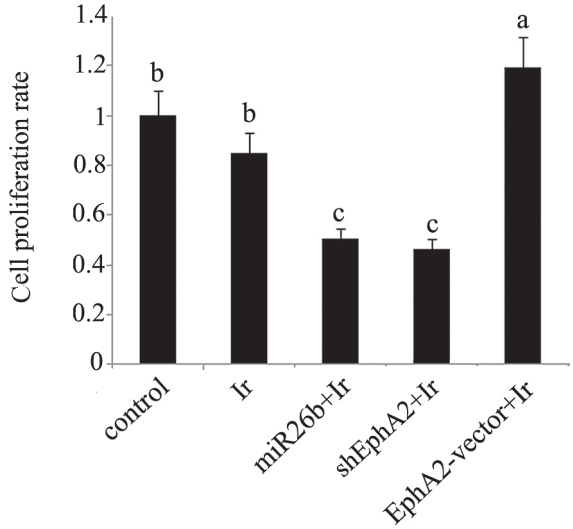
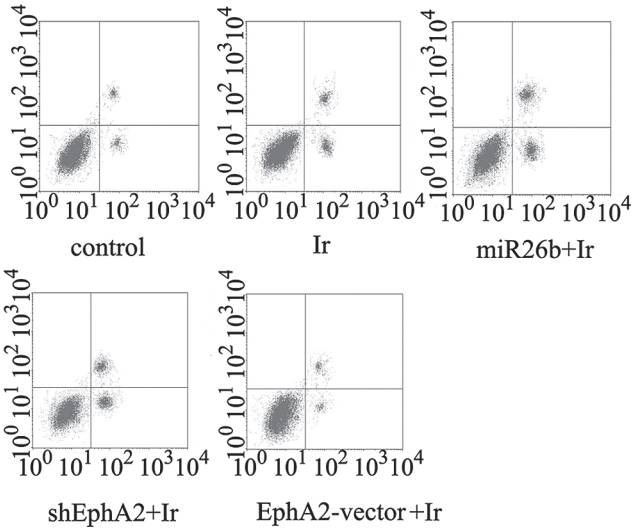


Fig. 5. Cell proliferation rates of 97H HCC cells following different treatments.
Before exposure to irradiation at dose of 1 Gy, 97H HCC cells were transfected with miR-26b mimics, EphA2-shRNA or EphA2 expression vector. Cell proliferation rate was tested using CCK-8 kits. Each bar represents the mean of three independent experiments. Bars not sharing a common letter differ ($P < 0.05$).
Ir, irradiation; miR-26b, miR-26b mimics; EphA2-vector, EphA2 over-expression vector; shEphA2, EphA2-shRNA.



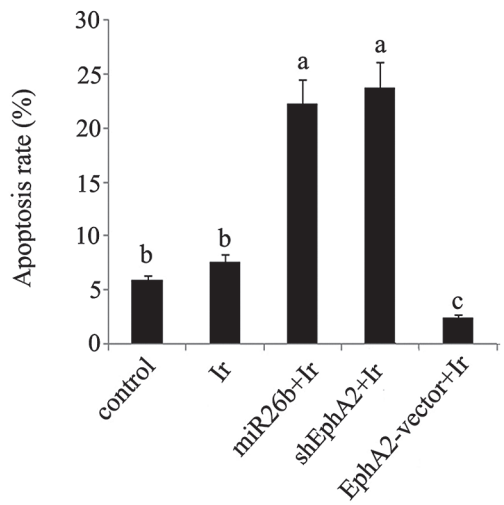


Fig. 6. Apoptosis of 97H HCC cells following different treatments.
Before exposure to irradiation at dose of 1 Gy, 97H HCC cells were separately transfected with miR-26b mimics, EphA2-shRNA and EphA2 expression vector. Apoptosis rate was tested by using Annexin V-fluorescein isothiocyanate/PI apoptosis kit and flow cytometer. The horizontal axes of the flow cytometer images represent Annexin V FITC, and the vertical axes represent PI. Each bar represents the mean of three independent experiments. Bars not sharing a common letter differ ($P < 0.05$).
Ir, irradiation; miR-26b, miR-26b mimics; EphA2-vector, EphA2 over-expression vector; shEphA2, EphA2-shRNA.

Related research showed that down-regulated miR-26b contributes to epithelial-mesenchymal transition and chemoresistance in HCC, which may be responsible for the relatively rapid malignant transformation and poor chemotherapeutic effect in HCC (Verghese et al. 2013; Shen et al. 2014). Our study assessed the effect of artificially elevating miR-26b expression in 97H HCC cells with lowest miR-26b expression among tested HCC cell lines, and evaluated the change of radiosensitivity. Enhanced miR-26b function effectively diminished post-irradiation cell proliferation rate and promoted irradiation-induced apoptosis, indicating that miR-26b enhances HCC radiosensitivity.

Furthermore, 97H HCC cells with the enhanced miR-26b function significantly decreased invasion ability under low-dose irradiation. According to clinical experience, cancer cells migrating to and invading other organs and tissues not only make it difficult to undertake RT, but also increase the possibility of bypassing irradiation attack (Liu et al. 2011). Thus, the miR-26b-mediated inhibition of the invasion of 97H HCC cells can, in turn, enhance the effect of RT. In this context, miR-26b decreased E-cadherin expression and increased vimentin expression (Shen et al. 2014), which may explain the miR-26b-induced attenuation of the invasion and migration ability of 97H HCC cells.

Our luciferase reporter assay, along with previous findings, verified that EphA2 mRNA is a target of miR-26b (Wu et al. 2011). Western blotting analysis indicated that miR-26b exerted inhibitory effect on endogenous EphA2 protein level in 97H HCC cells. It is been well established that miRNAs form a class of small noncoding RNAs that can negatively regulate gene expression by interacting with the 3′-UTR of protein-coding genes, further leading to translational inhibition (Shen et al. 2014). With regard to miR-26b, there is evidence that miR-26b silences the expression of ubiquitin-specific peptidase 9, X-linked (USP9X), TGFβ-activated kinase-1 (TAK1), TAK adaptors 3 (TAB3), tankyrase 1 binding protein 1 (TNKS1BP1), cleavage and

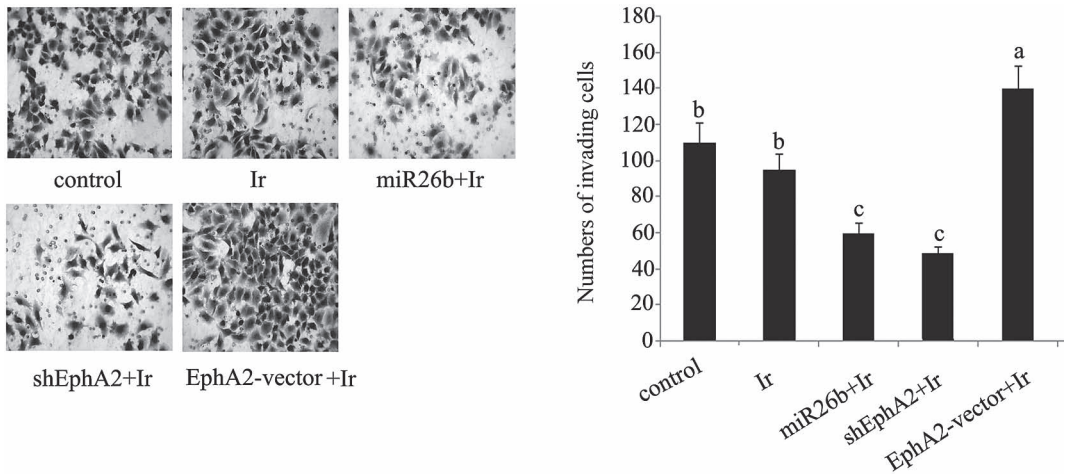


Fig. 7. Invasion ability of 97H HCC cells following different treatments.
Before exposure to irradiation at dose of 1 Gy, 97H HCC cells were transfected with miR-26b mimics, EphA2-shRNA or EphA2 over-expression vector. Cell invasion ability was tested using transwell chamber. Each bar represents the mean of three independent experiments. Bars not sharing a common letter differ ($P < 0.05$).
Ir, irradiation; miR-26b, miR-26b mimics; EphA2-vector, EphA2 over-expression vector; shEphA2, EphA2-shRNA.

polyadenylation-specific factor 7 (CPSF7), and collagen type XII alpha 1 (COL12A1), which could be the underlying reason for the increase in chemosensitivity and the inhibition of cell proliferation and migration (Verghese et al. 2013; Shen et al. 2014; Zhao et al. 2014).

To determine whether EphA2 expression influences the enhancing effect of miR-26b on the radiosensitivity of HCC, EphA2 was down-regulated or over-expressed by transfecting with EphA2-shRNA or EphA2 over-expression vector, respectively. Our results showed that the radiosensitivity of HCC cells is inversely correlated with EphA2 expression level, which indicates that EphA2 itself can influence radiosensitivity. The transfection with miR-26b mimics or EphA2-shRNA caused the similar decrease in EphA2 expression, which was associated with the decrease in cell proliferation rate and cell invasion ability and with the increase in apoptosis rate of cells exposed to low-dose irradiation. These results indicate that miR-26b enhances the radiosensitivity of 97H HCC cells mainly by targeting EphA2.

In summary, EphA2 was identified as a target of miR-26b by the luciferase reporter assay and confirmed to be inversely correlated with the radiosensitivity of HCC cells, on the basis of our finding that EphA2 over-expression and down-regulation decreased and increased the radiosensitivity of 97H HCC cells, respectively. Transfection with miR-26b mimics or EphA2-shRNA caused the similar degree of the decrease in EphA2 expression, which was associated with the increased radiosensitivity. This study has indicated that miR-26b enhances the radiosensitivity of HCC by targeting EphA2 protein. miR-26b and EphA2 are potential therapeutic targets to improve the therapeutic effect of low-dose RT on HCC.

Conflict of Interest

The authors declare no conflict of interest.

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