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MicroRNA-30c-1-3p IS A SILENCER OF THE PREGNANE X RECEPTOR BY TARGETING THE 3'-UNTRANSLATED REGION AND ALTERS THE EXPRESSION OF ITS TARGET GENE CYTOCHROME P450 3A4

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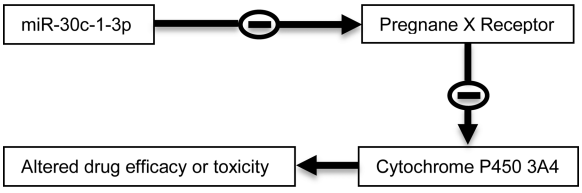
Abstract

The pregnane X receptor (PXR) is a master regulator of genes involved in drug elimination. Recently, activation of PXR has also been linked to the development of many disease conditions such as metabolic disorders and malignancies. MicroRNAs (miRs) emerge as important molecular species involved in these conditions. This study was undertaken to test a large number of miRs for their ability to regulate PXR expression. As many as 58 miRs were tested and miR-30c-1-3p was identified to suppress PXR expression. The suppression was achieved by targeting the 3'-untranslated region, 438 nucleotides from the stop codon. The suppression was detected in multiple cell lines from different organ origins. In addition, miR-30c-1-3p altered basal and induced expression of cytochrome P450 3A4 (CYP3A4), a prototypical target gene of PXR. The alteration varied depending on the time and amounts of miR-30c-1-3p. CYP3A4 is responsible for the metabolism of more than 50% medicines. The interconnection between miR-30c-1-3p and PXR signifies a role of miRs in drug-drug interactions and chemosensitivity.

Graphical abstract



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1. Introduction

All organisms are exposed constantly to toxic chemicals from both foreign and endogenous sources. Organisms such as humans have evolved several defensive systems against chemical insults [1]. In mammals, these systems are generally referred to as phase I [2, 3], phase II [4] and phase III [5, 6]. Phase I and II consist of drug-metabolizing enzymes, whereas phase III of drug transporters. The expression of these genes undergoes constant changes in response to chemical stimuli. The pregnane X receptor (PXR, NR1I2) is established as a master transcription factor intimately involved in the regulated expression of these genes [7–9]. Structurally, PXR belongs to the nuclear hormone receptor superfamily [10, 11]. Like other nuclear receptors, PXR consists of a variable N-terminal domain, a highly conserved DNA-binding domain, a hinge region and a multifunctional C-terminal ligand-binding domain [10]. The DNA-binding domain recognizes conical sequence AGG/TTCA [12, 13]. The major portion of the ligand-binding domain is helical in structure, and the C-terminal helix (helix 12) is directly involved in switching from repressing to activating status of a target gene [14]. Binding to an agonist induces conformational changes of this helix, leading to a platform favoring association with coactivators, namely transactivation.

The expression of PXR itself, like its target genes, is regulated by certain xenobiotics and disease conditions [8, 15–18]. For example, the hypolipidemic agent clofibrate and synthetic glucocorticoid dexamethasone have been shown to induce PXR [17, 18]. The induction synergistically increased the expression of cytochrome P450 3A genes (CYP3A) [17, 18], the prototypical targets of PXR [10]. Dexamethasone induced PXR in both rodents and humans [16, 18, 19]. Proinflammatory stimuli, on the other hand, have been shown to suppress the expression of PXR [20, 21]. The level of PXR mRNA was rapidly decreased in rodents treated with lipopolysaccharide, a potent immune stimulant [15]. In human hepatocytes, proinflammatory cytokine interleukin-6 markedly reduced the levels of PXR mRNA [20]. The suppression was accompanied by significant reduction of the induction of PXR-regulated genes such as CYP3A23 [15, 20, 21].

While transactivation and repression are recognized as major mechanisms for the regulated expression of PXR [17, 18, 20], post-transcriptional mechanisms have been increasingly implicated. MicroRNA (miR)-148a reportedly down-regulated PXR post-transcriptionally [22]. On the other hand, miRs are important regulators in a wide spectrum of diseases including malignances and metabolic disorders [23–25]. Interestingly, PXR has been linked to the development of these very conditions [26, 27]. Expression of constitutively activated PXR markedly increased hepatic lipids in mice and the steatotic phenotype was recapitulated in human primary hepatocytes by rifampicin, a prototypical activator of human PXR [26]. The expression of PXR is dysregulated in a wide range of cancerous tissues such as breast cancer, endometrial cancer and colon cancer [27].

In this study, we took a comprehensive approach and tested a large number of miRs for their ability to regulate PXR expression. As many as 58 miRs were tested and miR-30c-1-3p was identified to suppress PXR. The suppression was achieved by targeting the 3’-untranslated region (UTR) and detected in multiple cell lines from different organ origin. Importantly, miR-30c-1-3p was shown to alter the expression of CYP3A4, a prototypical target gene of

PXR. CYP3A4 is responsible for the metabolism of more than 50% medicines. Therefore, the PXR and miR-30c-1-3p connection likely constitutes a major determinant in drug-drug interactions and chemosensitivity.

2. Materials and methods

2.1. Plasmid constructs

All miR precursor clones were purchased from System Biosciences Inc (Mountain View, CA). The CYP3A4-DP-Luc reporter was described in our previous publication [28]. The PXR cDNA reporters harboring a 3'-UTR segment were prepared with the pGL3 promoter vector (Promega, Madison, WI) through Xba I and Fse I restriction endonuclease sites. The 3'-UTR segments were amplified by PCR with high fidelity Platinum *Taq* DNA polymerase (Life Technology Co., Carlsbad, CA). A cDNA clone encoding human PXR, used as the PCR template, was described elsewhere [16]. The PXR 3142/3691Luc and 3690/4408Luc reporters were prepared initially and the PXR 3142/3691Luc reporter was used as the template for preparing deletion mutants at 5' end. The primers for PCR amplification are listed in Table I. All reporter constructs were subjected to sequence analysis.

2.2. Cell transfection and luciferase assay

Three cell lines were used in this study including 293T (human embryonic kidney), HepG2 (human hepatocellular carcinoma) and LS180 (human colon adenocarcinoma). HepG2 and LS180 lines were purchased from American Type Culture Collection (Manassas, VA), but the 293T line was from GenHunter Corporation (Nashville, TN). All cell lines were maintained in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum, penicillin and streptomycin, 1× non-essential amino acids. Unless otherwise indicated, cells were plated in 48 well-plates and transiently transfected by GenJet version II from SignaGen Laboratories (Rockville, MD). For reporter assays, the transfection mixture typically contained 50 ng of a reporter, 50 ng of a miR construct and 0.2 ng of the CMV-*Renilla* luciferase plasmid. After incubation at 37°C for 24 h, cells were extensively washed, collected and assayed for luciferase activities with the Dual-Luciferase Reporter Assay System as described previously [6, 16]. The reporter luciferase activity was normalized with *Renilla* luciferase activity, and the vector-transfected cells served as the basal reporter activity for miRs-transfected cells. It should be noted that the same amount of total plasmids were used in all reporter assays.

2.3. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

The LS180 cell line was primarily used for the RT-qPCR because this cell line has been shown to robustly support PXR-directed transactivation [29]. Cells were typically plated in 24-well plates for overnight and then transfected with the miR-30c-1-3p construct or the corresponding vector. Cells were harvested 72 or 96 h after the transfection. In some cases, cells were treated with DMSO or rifampicin (10 μM) after transfection. Harvested cells were used for the preparation of total RNA. For the determination of PXR or CYP3A4 mRNA, total RNA (1 μg) was subjected to the synthesis of the first strand cDNA as described previously [30]. cDNAs were then diluted 8 times and RT-qPCR was conducted with *TaqMan* Gene Expression Assay (Applied Biosystems, Foster City, CA). The *TaqMan*

probes were: PXR, Hs00243666_m1; CYP3A4, Hs00604506_m1; glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 4352934E; and RNA polymerase II, Hs00172187_m1. The PCR amplification was conducted in a total volume of 20 μl containing universal PCR master mixture (10 μl), gene-specific *TaqMan* assay mixture (1 μl), and cDNA template (6 μl). The mRNA levels were normalized according to the level of GAPDH and the normalization of selected samples was confirmed based on the signal of RNA polymerase II. Amplification and quantification were done with the Applied Biosystems 7500 Real-Time PCR System.

3. Results

3.1. Identification of miR-30c-1-3p as a suppressive miR of PXR

PXR is a master regulator of the expression of genes involved in drug metabolism, metabolic disorders and tumor growth behaviors [7–9, 26, 27]. Like its target genes, the expression of PXR is regulated by factors such as age, disease mediators and therapeutic agents [17, 18, 31, 32]. While transactivation and repression are common mechanisms in regulated expression of PXR, emerging evidence suggests an involvement of post-transcriptional mechanisms in PXR expression, particularly through miR species [22]. miRs constitute a superfamily of small RNA species, and many of them are implicated in the development of a wide range of diseases [23–27]. To shed light on the missing link between PXR expression and miRs, we tested a large number of miRs for their ability to regulate the expression of PXR. We took the advantage of miRs as predominantly post-transcriptional regulators [24, 25], mRNA-based PXR reporters (cDNA reporters) were used for the initial study.

While there are exceptions, miRs usually target the 3’-UTR sequences. Therefore, we constructed two luciferase reporters that together harbor the entire 3’-UTR sequence of the dominant human PXR transcripts (Fig. 1A) (NM_003889). These two reporters were designated PXR3142/3691Luc and PXR3690/4408Luc, respectively. These two reporters were prepared by ligating the respective 3’-UTR fragments into the XbaI and FseI sites. It should be noted that the full-length 3’ UTR region of PXR has an XbaI site, and two reporters were prepared to avoid internal XbaI site. Cotransfection was performed in 293T cells to determine the effect of a miR on the reporter activity. In addition, a *renilla* luciferase construct was included in the transfection. As shown in Fig. 1B, all miRs, with an exception of miR-30c-1-3p, affected the activity of both reporters to a similar extent. Transfection of miR-30c-1-3p, compared with the vector, resulted in decreased activity of PXR3142/3691Luc by 50%, but only 10% decrease on the activity of PXR3690/4408Luc. Some miRs such as miR-26A-2, compared with the vector, caused significantly increases in the activity of both reporters. Others such as miR-433 and miR-127 significantly decreased the activity of both reporters (Fig. 1B). These miRs were not investigated further because of the following reasons: (1) they showed no selectivity toward two reporters, (2) bioinformatics analysis predicted no binding sites for these miRs, and/or (3) they affected the *renilla* luciferase activity, which was used for normalizing transfection efficiency.

3.2. Sequence-specific targeting by miR-30c-1-3p in both hepatic and intestinal cell lines

The screening study clearly demonstrated that human PXR mRNA is a sequence-specific target of miR-30c-1-3p. To locate the sequence that supports the action of this miR, a serial of deletions from the 5' end were made on the PXR3142/3691Luc reporter and the resultant reporters were tested for the lost activity toward miR-30c-1-3p. As shown in Fig. 2A, all deletion mutants, except PXR3592Luc and PXR3642Luc, were repressed by miR-30c-1-3p. These results established that this 50 base sequence, namely from 3542 to 3592 support the repression of miR-30c-1-3p on PXR expression. We are in the process of specifying the precise sequence and nucleotides that support the action of miR-30c-1-3p by site-directed mutagenesis.

It is well known that some miRs are processed in a cell-specific manner [33]. We next tested whether the repression by miR-30c-1-3p occurs in HepG2 and LS180 cell line. HepG2 was derived from hepatocellular carcinoma whereas LS180 from colon adenocarcinoma. Importantly, both organs abundantly express PXR [34, 35]. In addition, the cotransfection was performed with various amounts of miR-30c-1-3p to establish the concentration-response relationship. As expected, the PXR3542Luc but not PXR3592Luc reporter was repressed in both cell lines (Fig. 2B). HepG2 cells supported greater repression than LS180 cells. Overall, the magnitude of the repression occurred in a concentration-dependent manner (Fig. 2B).

3.3. Effect of miR-30c-1-3p on the expression of CYP3A4

The study with the reporters established that the action of miR-30c-1-3p on PXR suppression is sequence-specific [24, 25]. Next we tested whether the repressive activity of the reporter can be recapitulated on the expression of the endogenous gene of PXR. LS180 but not HepG2 cells were used for the induction study because LS180 cells supported higher induction of CYP3A4 than HepG2 cells. Initially, cells were transfected with miR-30c-1-3p or the corresponding vector, cultured for various lengths of time, and the levels of PXR mRNA were determined. Fig. 3A shows representative results of this study. The level of PXR mRNA was decreased in miR-30c-1-3p transfected cells, and the 96 h time-point showed a relatively less decrease than the 72 h time-point (Left of Fig. 3A). The decrease in cells transfected with 20 ng plasmid of miR-30c-1-3p was less profound than that in cells transfected with 200 and 800 ng. Nonetheless, 200 and 800 ng caused a comparable decrease in both time-points. Next we tested whether miR-30c-1-3p alters the mRNA level of CYP3A4, a prototypical target of PXR [36]. While miR-30c-1-3p caused changes in the level of CYP3A4 mRNA, the changes varied depending on the amount of miR-30c-1-3p plasmid used for the transfection as well as the time after the transfection (Fig. 3A, Right). At the 72 h time-point, cells transfected with the miR-30c-1-3p plasmid at 20 and 200 ng exhibited increases in CYP3A4 mRNA, whereas a slight decrease was detected in cells transfected with 800 ng. At the 96 h time-points, significant decreases were detected in cells transfected with 200 and 800 ng plasmid (Fig. 3A, Right).

We next tested whether miR-30c-1-3p also alters the induction of CYP3A4. Cells were transfected with miR-30c-1-3p or the vector, and then treated with DMSO or rifampicin, a prototypical activator of human PXR. As shown in Fig. 3B (Left), the overall expression of

CYP3A4 mRNA was decreased at both basal and induced level with an exception of the basal level in cells transfected with 20 ng plasmid of miR-30c-1-3p. As described above, marked decreases in basal expression were detected in cells transfected with miR-30c-1-3p at 200 and 800 ng. As a result, these cells showed no changes in terms of fold of induction. To further establish the role in reduced induction of CYP3A4, a CYP3A4 reporter was tested for the reduced activation in response to miR-30c-1-3p [28]. Consistent with the result on the level of CYP3A4 mRNA, cotransfection of miR-30c-1-3p decreased the activation of this reporter by as much as 60% (Fig. 3C).

4. Discussion

PXR has been established to play a major role in the expression of genes involved in drug elimination [7, 9, 11]. However, emerging evident has suggested that this nuclear receptor, in addition to drug elimination, is integrally connected with endobiotic signaling and homeostasis of energy balance [11]. While PXR is recognized as a master regulator of gene expression, we and other investigators have reported that the expression of PXR is regulated by many factors such as age, disease mediators and therapeutic agents [17, 18, 31, 32]. Multiple mechanisms are reportedly involved in the regulated expression of PXR including transactivation, repression and miR-148a silencing [22]. This study identified and characterized miR-30c-1-3p as a silencer of PXR. A set of experiments have shown that miR-30c-1-3p targeted the PXR 3’-UTR and decreased the level of PXR mRNA, suggesting that miR-30c-1-3p downregulates PXR expression, at least partially by reducing the mRNA stability of this nuclear receptor. Importantly, this miR altered the expression of CYP3A4, a prototypical target of PXR [36], pointing to a functional role of miR-30c-1-3p in drug elimination.

It has been reported that miR-30c-1-3p belongs to the miR-30 family, and the human genome has six miR-30 genes [37, 38]. However, these genes produce only five distinct mature guide strands. Importantly, these guide strands have the identical seed sequence, allowing them to regulate the expression of the same target genes, at least through the guide strand. This study, however, has demonstrated that miR-30a, a member of this family, failed to suppress the PXR reporters (Fig. 1B), suggesting that PXR is a target of the passenger strand (i.e., miR-30c-1-3p). In addition to miR-30c-13p, the miR-30c class has another member, namely miR-30c-2-3p [39]. Both miRs are derived from intronic sequences of other genes. The miR-30c-1-3p gene is located at chromosome 1 and the miR-30c-2-3p gene is located at chromosome 6. Importantly, miR-30c-13p and miR-30c-2-3p differ in the sequence (Fig. 4A). Interestingly, miR-30c-2-3p matches better than miR-30c-1-3p with the PXR 3-UTR region (Fig. 4A). Therefore, it is likely that miR-30c-2-3p is more potent than miR-30c-1-3p in silencing PXR, although the relative expression of these two miRs likely determines their contribution to PXR silencing. In addition, miR-148a, another miR, reportedly silenced PXR [22]. Based on the recognition sequences (Fig. 4B), both miR-30c and miR-148a target the same RNA species of PXR, although their recognition elements are 218 nucleotides apart (Fig. 4B). Nevertheless, it remains to be determined whether and how miR-148a networks with miR-30c in terms of regulating PXR expression.

The silencing of PXR by miR-30c-1-3p may have important clinical consequences. In this study, we have shown that miR-30c-1-3p decreased the level of PXR mRNA accompanied by altered expression of CYP3A4, a prototypical gene of PXR [36]. The altered expression of CYP3A4, however, varied depending on time and the amounts of miR-30c-1-3p. The overall expression of CYP3A4 mRNA was decreased in both basal and induction conditions (Fig. 3B, Left), but the decrease was not evident until later time-point (Fig. 3A, Right). It has been reported that miR-mediated suppression was delayed [40]. The altered expression of CYP3A4 by miR-30c-1-3p was in particular as it represented a mechanism secondarily to the suppression of PXR. Such an indirect mechanism required longer time to achieve the anticipated effect. On the other hand, the basal level of CYP3A4 mRNA at the early time-point was actually increased when small amounts of miR-30c-1-3p were used (Fig. 3A, Right). The precise mechanism on the increase remains to be determined. Nuclear receptors including PXR are known to interact with co-repressors and coactivators [14]. It is the presence of a ligand that induces conformational changes from repressing to activating status of a target gene. It should be not emphasized that our observation, increased expression of CYP3A4 by PXR knockdown, was consistent with previous report that knockout of pxr (in mice) increased the expression of cyp3a11 (the counterpart of human CYP3A4) [41].

The functionality of PXR has been linked to a wide range of behavior changes of malignancies and some of them are opposing to each other [27]. In some cases, activation of PXR up-regulates the expression of proapoptotic genes thus shows anti-tumor activity. In other cases, PXR is linked to upregulation of antiapoptotic genes and favors tumor progression. Nevertheless, up-regulated expression of genes involved in drug eliminations is generally considered to be a major contributing factor to the development of chemoresistance. Interestingly, miR-30c has been shown to promote cell apoptosis, inhibit cell proliferation, reduce tumor clonogenicity and suppress metastatic potentials [42, 43]. In addition, the expression of miR-30c was significantly decreased in many chemoresistant cell lines [44]. It remains to be determined whether miR-30c members can overcome PXR-directed chemoresistance. Interestingly, miR-148a, another silencer of PXR, has also been downregulated in advanced cancer [45].

In summary, our study presents several important conclusions. Firstly, identification of miR-30c-1-3p as a negative regulator of PXR, along with the previous reporter on miR-148a, points the existence of miR-networks in regulating the functionality of PXR. Secondly, miR-30c-1-3p but not miR-30a suppressed PXR, underscoring the importance of the passenger strand in gene silencing. Thirdly, activation of PXR has been closely linked to the development of a spectrum of tumor behaviors, particularly in chemoresistance. miR-30c and miR-148a, on the other hand, have been associated with less aggressive behaviors of malignancy. These findings suggest that miR-30c/miR-148a-PXR represents favorable outcomes of chemotherapy, particularly chemotherapeutic agents with a potent activating activity of PXR.

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Abbreviation

CYP3A4	cytochrome P450 3A4
DMEM	Dulbecco's modified eagle medium
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
miR	microRNA
PXR	pregnane X receptor
RT-qPCR	reverse transcription-quantitative polymerase chain reaction
UTR	untranslated region

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Highlights

1. The pregnane X receptor (PXR) is a master regulator of genes for drug metabolism.
2. miR-30c-1-3p is a silencer of PXR by targeting the 3’ untranslated region.
3. The silencing led to decreased induction of cytochrome P450 3A4 (CYP3A4).
4. CYP3A4 is involved in the metabolism of more than half of therapeutic agents.

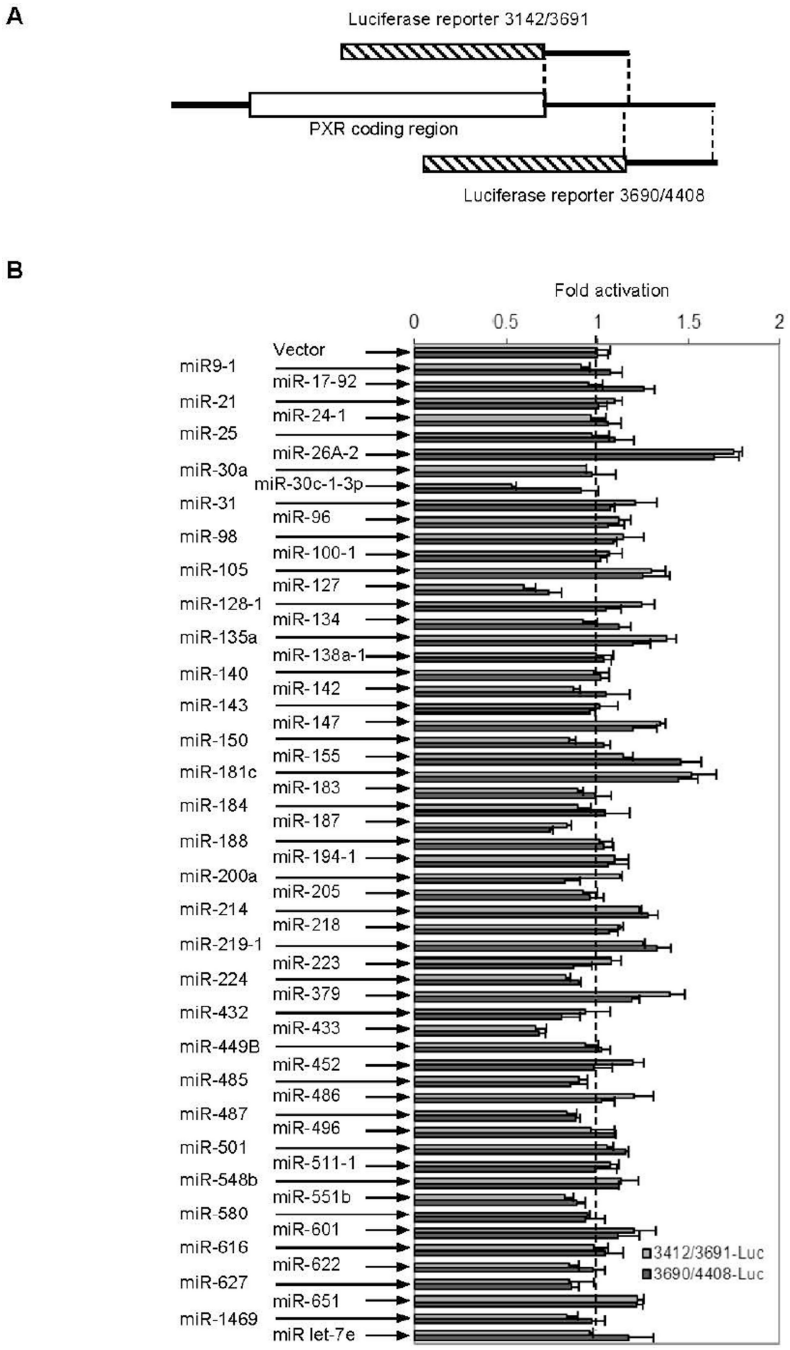

Fig. 1. Suppression of PXR reporters derived from the 3'-untranslated region (UTR)

(A) Diagrammatic presentation of PXR reporters The PXR3142/3691Luc reporter contains the cDNA segment from nucleotide 3142 to 3691, whereas the PXR3690/4408Luc reporter contains from nucleotide 3690 to 4408. The hatched box represents the luciferase coding sequence. (B) Identification of PXR suppressive miR(s) Cells (293T) were transiently transfected by GenJet version II with a mixture containing 50 ng of a miR precursor construct, 50 ng of a reporter, or the vector along with 5 ng of the *Renilla* luciferase plasmid. The transfected cells were cultured for 24 h, harvested and analyzed for luciferase activities

with a Dual-Luciferase Reporter Assay System. The results were from one of two experiments in triplicate.

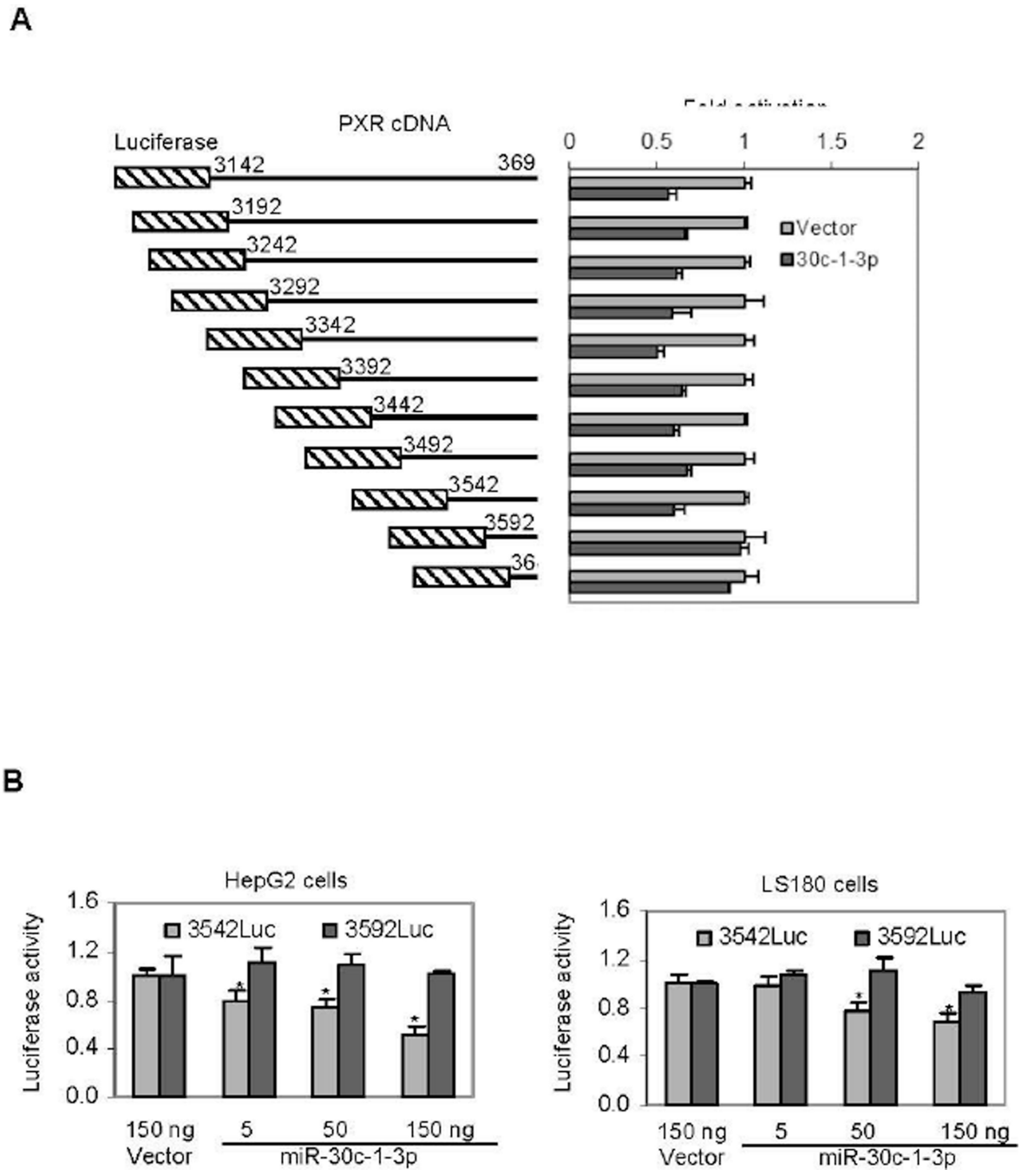

Fig. 2. Characterization of miR-30c-1-3p on the suppression of PXR

(A) Dissection of the PXR3142/2691Luc reporter for the identification of miR-30c-1-3p response Cells (293T) were transiently transfected by GenJet version II with a mixture containing 50 ng of the miR-30c-1-3p construct, 50 ng of a reporter, or the vector along with 5 ng of the *Renilla* luciferase plasmid. The transfected cells were cultured for 24 h, harvested and analyzed for luciferase activities with a Dual-Luciferase Reporter Assay System. (B) Suppression of PXR3542/3691Luc by miR-30c-1-3p in HepG2 and LS180 cell lines Cells were transfected by GenJet version II with a mixture containing 5-150 ng of the

miR-30c-1-3p construct, 50 ng of a reporter (PXR3542/3691 or PXR3592/3691Luc, or the vector along with 5 ng of the *Renilla* luciferase plasmid. The vector plasmid was used to equalize the total amount of constructs. The transfected cells were cultured for 24 h, harvested and analyzed for luciferase activities as described above. Asterisk signs indicate statistical significance from vector-transfected cells ($P < 0.05$). Significant differences were made according to One-way ANOVA followed by a DUNCAN's multiple comparison test.

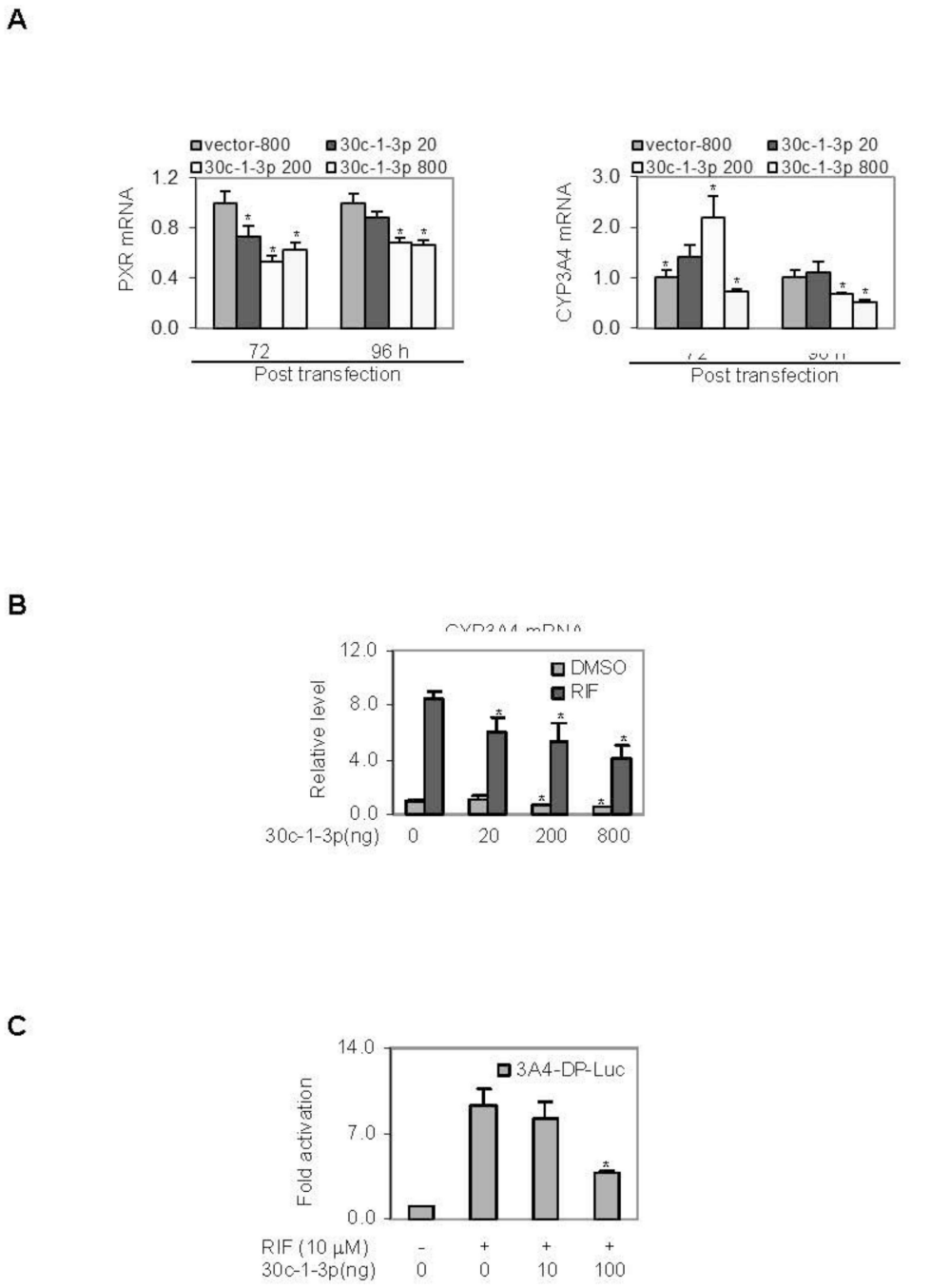

Fig. 3. Effect of miR-30c-1-3p on the expression of CYP3A4

(A) Effect of miR-30c-1-3p on the expression of PXR and CYP3A4 Cells (LS180) were transfected by GenJet version II with the miR-30c-1-3p construct at 20, 200 and 800 ng. The vector plasmid was used to equalize the total amount of constructs. Cells were harvested 72 or 96 h post-transfection. Total RNA was isolated and the mRNA levels of PXR (Left) and CYP3A4 (Right) were determined by RT-qPCR. All experiments were performed three times in triplicate. Asterisk signs in the data indicate statistical significance from vector-transfected cells ($P < 0.05$). (B) Effect of miR-30c-1-3p on the induction of CYP3A4 Cells

(LS180) were transfected as described above and treated with DMSO or 10 μM rifampicin (RIF) 72 h post-transfection. The treated cells were collected 24 h thereafter and the level of CYP3A4 mRNA was determined by RT-qPCR. All experiments were performed three times in triplicate. Asterisk signs in the data indicate statistical significance from vector-transfected cells or cells treated with DMSO ($P < 0.05$). (C) Effect of miR-30c-1-3p on the activation of CYP3A4-DP-Luc reporter Cells (LS180) were plated in 48 well-plates and transfected with a mixture containing 50 ng of the CYP3A4-DP-Luc reporter, 50 ng of miR-30c-1-3p or the vector along with 5 ng of the *Renilla* luciferase plasmid. The vector was used to equalize the total amount of constructs. After incubation at 37°C for 24 h, the transfected cells were treated with 10 μM rifampicin (RIF) or the same volume of DMSO for 48 h. Luciferase activities were determined with a Dual-Luciferase Reporter Assay System and the reporter activity was normalized based on the *Renilla* luminescence signal. All experiments were performed three times in triplicate. Asterisk signs in the data indicate statistical significance from vector-transfected and RIF-treated cells ($P < 0.05$).

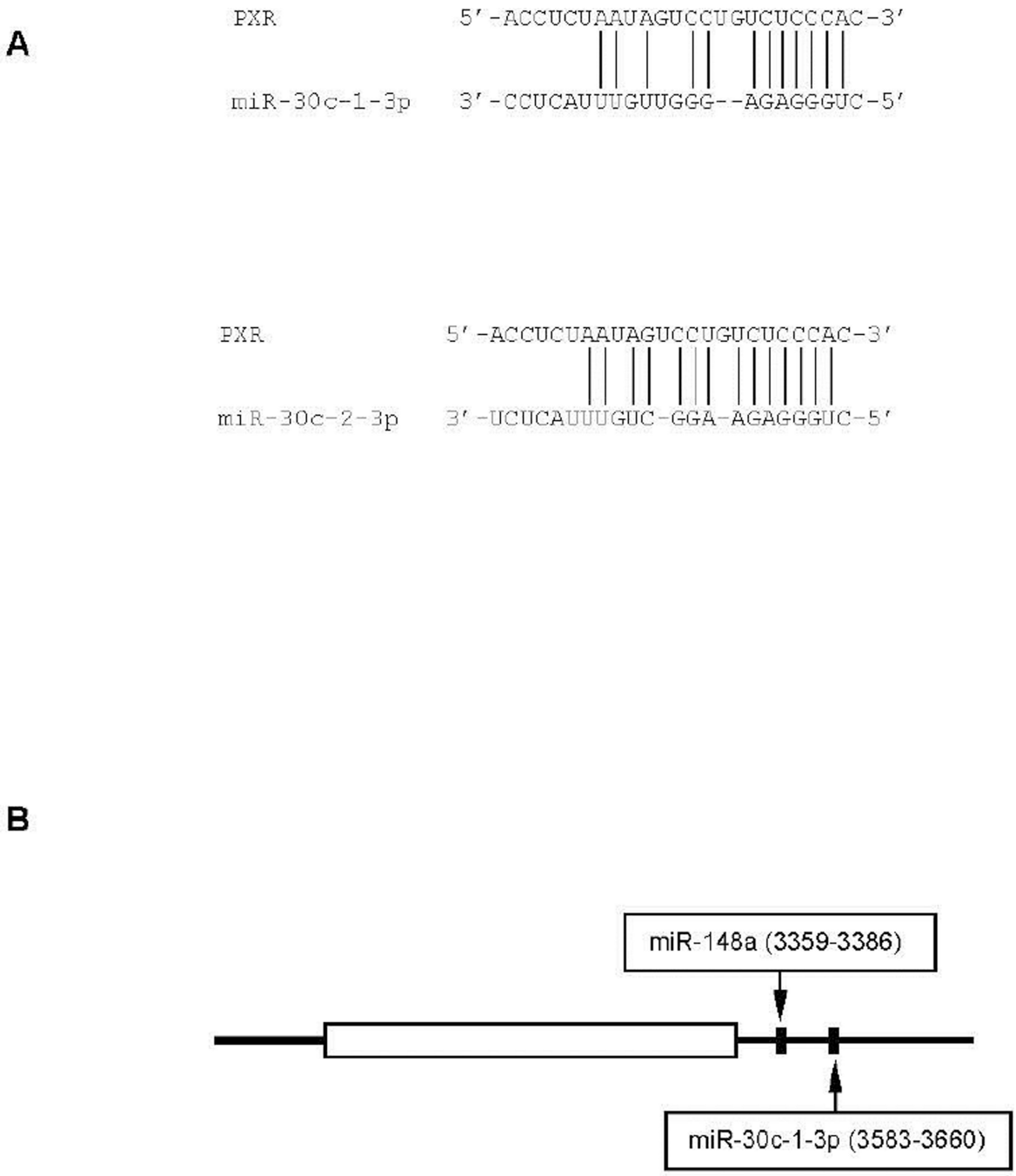

Fig. 4. Sequence targeted by miR-30c-1-3p and its location in the 3'-UTR of PXR transcript

(A) Sequence targeted by mir-30c The sequence targeted by miR-30c is shown as pair-matching for both miR-30c-1-3p and miR-30c-2-3p. (B) Location of the sequence targeted by miR-30c and miR-148a In addition to the location of the sequence targeted by miR-30c, the location of the sequence targeted by miR-148a is also shown. Specifically, miR-30c (both 30c-1-3p and -2-3p) likely targets the sequence from nucleotide 3583 to nucleotide

3660, whereas miR148a from 3359 to 3386, respectively. The sequence is numbered according to NM_003889.

Table I

Sequences of Oligonucleotides

Oligonucleotide	Sequence
PXR3142/3691Luc (XbaI)	5’-tgagcggctgcccttggg-3’
PXR3142/3691Luc (FseI)	5’-agaggactcccacagata-3’
PXR3690/4408Luc (XbaI)	5’-ggagtcctctagagagatgagaagccagga-3’
PXR3690/4408Luc (FseI)	5’-gtacattatttaattcct-3’
PXR3192/3691Luc (XbaI)	5’-gccctctgagccgccact-3’
PXR3242/3691Luc (XbaI)	5’-gacaatgccctgctggcc-3’
PXR3292/3691Luc (XbaI)	5’-ggctagcattcctcagga-3’
PXR3342/3691Luc (XbaI)	5’-ctgtagggagtgaagcca-3’
PXR3392/3691Luc (XbaI)	5’-aggtcaggaccatcagag-3’
PXR3442/3691Luc (XbaI)	5’-tgtggtctggggagaaat-3’
PXR3492/3691Luc (XbaI)	5’-aagggaccaagcgaccaa-3’
PXR3542/3691Luc (XbaI)	5’-ccacgtttgttcgcttcc-3’
PXR3592/3691Luc (XbaI)	5’-gtctcccacttcccactc-3’
PXR3642/3691Luc (XbaI)	5’-tccaggcctgtactcatc-3’

Numbered according to NM_003889.