STAT3 upregulates miR-92a to inhibit RECK expression and to promote invasiveness of lung cancer cells

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Background: Signal transducer and activator of transcription 3 (STAT3) activation is frequently found in human lung cancer and is associated with increased metastasis and reduced survival. How STAT3 enhances invasiveness is unclear.

Methods: The expression of microRNAs and target genes was measured by real-time RT–PCR. Protein level was studied by western blotting. Luciferase reporter assay was used to confirm the direct targeting of microRNAs. Gelatin zymography was used to study matrix metalloproteinase (MMP) activity. Transwell assay was used to investigate cell migration and invasion.

Results: Enforced expression of STAT3 decreases the endogenous MMP inhibitor RECK protein but not mRNA level in H460 cells. Conversely, STAT3 inhibitor S3I-201 increases RECK protein in STAT3-activating H1299 cells. We demonstrate that STAT3 upregulates miR-92a to repress RECK via post-transcriptional inhibition. The RECK 3′-untranslated region (3′UTR) reporter activity assay suggests that RECK is a direct repression target of miR-92a. Delivery of pre-miR-92a reduces RECK protein level whereas transfection of anti-miR-92a restores STAT3-induced downregulation of RECK. Anti-miR-92a attenuates MMP activity, migration and invasion of H1299 cells and STAT3-overexpressing H460 cells, suggesting miR-92a is critical for STAT3-induced invasiveness.

Conclusion: The STAT3-induced miR-92a promotes cancer invasion by suppressing RECK and targeting of the STAT3/miR-92a axis may be helpful for cancer treatment.

In lung cancer, signal transducer and activator of transcription 3 (STAT3) activation is important for tumour development and promotion (Song *et al*, 2003; Yeh *et al*, 2006). The STAT3 is a cytoplasmic transcription factor that can be phosphorylated and activated by growth factors and cytokines. Active STAT3 proteins form homodimer or heterodimer with other transcription factors and translocate into the nucleus, where they bind to specific promoters to induce the expression of target genes like cyclin D1 and Bcl-xl (Bowman *et al*, 2000; Buettner *et al*, 2002; Leslie *et al*, 2006). Activation of STAT3 is frequently found in human lung cancer and is associated with increased metastasis and reduced survival (Haura *et al*, 2005; Cortas *et al*, 2007). Recent studies demonstrate that STAT3 stimulates the transcription of matrix metalloproteinase-2 (MMP-2) in melanoma cells to enhance tumour invasion and brain metastasis (Xie *et al*, 2004). In bladder cancer cells, STAT3 is involved in the induction of MMP-1 by epidermal growth factor (Itoh *et al*, 2006). Constitutively activated STAT3 also increases MMP-9 activity to enhance the transformation of human mammary epithelial cells (Dechow *et al*, 2004). These results collectively suggest that upregulation of the activity of MMPs is critical for STAT3-induced invasion and metastasis. However, it should be noted that *in vivo* MMP activity is controlled by the balance between MMPs and their inhibitory proteins including tissue inhibitor of metalloproteinases and Reversion-inducing Cysteine-rich protein with Kazal motifs (RECK) (Noda *et al*, 2003).

The *RECK* gene was first identified as a tumour metastasis suppressor, which induced morphological reversion in v-Ki-Ras-transformed NIH/3T3 cells (Takahashi *et al*, 1998). Subsequent studies show that RECK is an endogenous MMP inhibitor and participates in embryonic development (Oh *et al*, 2001; Noda *et al*, 2003; Meng *et al*, 2008; Noda *et al*, 2010). The expression of RECK is found in most normal human tissues and untransformed cells; however, it is undetectable in many cancers (Masui *et al*, 2003; Takeuchi *et al*, 2004). In addition, RECK downregulation is strongly associated with poor prognosis and short survival. We have elucidated the molecular mechanisms by which RECK expression is inhibited by different oncogenes including latent membrane protein 1 (LMP-1) of Epstein–Barr virus (Liu *et al*, 2003), Ras (Chang *et al*, 2004) and Her-2/Neu (Hsu *et al*, 2006). Our data indicate that epigenetic regulation via DNA methylation and histone deacetylation is important for the inhibition of RECK gene transcription by these oncogenes (Chang *et al*, 2004, 2006). However, only 50–60% of the lung tumour tissues exhibit RECK promoter methylation, suggesting that other inhibitory mechanisms are involved in the downregulation of RECK in lung cancer (Cho *et al*, 2007).

MicroRNAs (miRNAs) are endogenous 21–24-nucleotide single-stranded noncoding RNAs that can bind to the 3′-untranslated region (3′UTR) of target mRNAs to induce translation repression or mRNA cleavage and to attenuate protein expression (Esquela-Kerscher and Slack, 2006). Recent studies indicate that miRNAs play important roles in either tumour suppression or promotion (Chan *et al*, 2005; Iorio *et al*, 2005; Ma *et al*, 2007). Interestingly, RECK has been demonstrated to be a repression target of several miRNAs including miR-21, miR-372/373, miR-222, miR-7 and miR-182 (Gabriely *et al*, 2008; Hu *et al*, 2008; Zhang *et al*, 2008; Loayza-Puch *et al*, 2010; Hirata *et al*, 2012; Jung *et al*, 2012; Li *et al*, 2012). Because STAT3 can activate the expression of several miRNAs in various cancers like multiple myeloma, colon cancer and head and neck cancer (Loffler *et al*, 2007; Iliopoulos *et al*, 2010; Bourguignon *et al*, 2012), we study whether STAT3 could inhibit RECK via miRNA to enhance invasiveness of lung cancer cells.

MATERIALS AND METHODS

Cell lines and reagents. The H460, H1299 and H358 human lung cancer cell lines were obtained from the cell bank of National Health Research Institute (Miaoli, Taiwan). Anti-RECK and anti-STAT3 antibodies were purchased from BD Transduction Laboratories (Franklin Lake, NJ, USA). Anti-phospho-STAT3, anti-Cyclin D1 and anti-integrin α5 were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-actin and anti-FLAG were purchased from Sigma-Aldrich (St Louis, MA, USA). MiR-92a precursor (pre-miR-92a), anti-miR-92a and negative control miRNA were obtained from Ambion (Austin, TX, USA). The STAT3 inhibitor S3I-201 was obtained from Merck (Rockland, MA, USA). Effectene transfection reagent was purchased from QIAGEN (Valencia, CA, USA). Constitutively active STAT3 expression vector RcCMV-S3C was kindly provided by Dr HS Liu (National Cheng Kung University, Tainan, Taiwan).

Establishment of STAT3-activating stable cell lines. The H460 cells were transfected with RcCMV-S3C by Effectene transfection reagent for 72 h and cultured with medium containing G418 $(0.5 \, mg \, ml^{-1})$ for 3 weeks. The stable clone with highest STAT3 activity (indicated by phospho-STAT3 level) generated by G418 selection was used for subsequent experiments.

RT–PCR and real-time RT–PCR. RNA (1 μg) isolated from cells was used to synthesise cDNA by using the NCode VILO miRNA cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). The mRNA

and miRNA levels were quantified by using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) system. The primers used are RECK forward 5′-TCTGCAGGGGAAGTTGGTTG-3′; RECK reverse 5′-CAGTTACAGGGCAGACCTGT-3′; miR-92a 5′-CACT TGTCCCGGCCTGT-3′; and miR-21 5′-CGGTAGCTTATCA GACTGATGTTGA-3′. Small nuclear RNA U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as internal controls for miRNA and mRNA quantification. Total RNA was also subjected to RT–PCR analysis as described previously (Chang *et al*, 2006).

Western blot analysis. Cells were transfected with the pre-miRNA and anti-miRNA for 72 h. Cellular proteins were harvested and equal amount of proteins was subjected to SDS–PAGE. Proteins were transferred to nitrocellulose membranes and the blots were probed with different primary antibodies and developed by enhanced chemiluminescence reagents as described previously (Hsu *et al*, 2008).

Luciferase reporter assay. Control reporter and 3′-UTR reporter plasmid of *RECK* gene was purchased from GeneCopoeia (Rockville, MD, USA). Reporter plasmids were transfected into cells. After 48 h, luciferase activity was determined and normalised to *Renilla* luciferase activity as previously described (Chang *et al*, 2006).

In vitro invasion and migration assay. *In vitro* invasion and migration assays were performed by using transwell units with polycarbonate filters (pore size 8 μm). The filters were coated with Matrigel (Discovery Labware) on the upper side for invasion assay. Cells were transfected with the anti-miR-92a for 48 h. After that, 5000 cells in 100 μl of medium were seeded onto upper well and allowed to invade for 24 h. After incubation, cells on the upper part of the membrane were removed with a cotton swab. Invaded cells on the bottom surface of the membrane were fixed in formaldehyde, stained with Giemsa solution and counted under a microscope.

Gelatin zymography. Cells were transfected with the anti-miR92a for 48 h and incubated with serum-free medium for another 24 h. Conditioned medium was harvested and zymography assay was done as described previously (Liu *et al*, 2003).

Statistical analysis. Data were analysed using a two-tailed Student's *t*-test. Differences with a *P*-value of < 0.05 were considered as significant.

RESULTS

STAT3 activation negatively regulates RECK expression in human lung cancer cell lines. Expression of STAT3 and RECK in different lung cancer cell lines was studied. As shown in Figure 1A, STAT3 was constitutively activated (as indicated by phospho-STAT3) in H1299 and H358 and the RECK protein level was very low in these two cell lines. Conversely, H460 cells exhibited low STAT3 activity and high level of RECK protein (Figure 1A). The ratio of phospho-STAT3/STAT3 of H460 cells was reduced to 70% of that of H1299 cells. Our previous results have demonstrated that transcription of *RECK* gene was downregulated in H358 cells via promoter methylation (Chang *et al*, 2006; Cho *et al*, 2007). This conclusion was reconfirmed by the observation that RECK mRNA level was very low in this cell line (Figure 1A). However, RECK mRNA level of H1299 and H460 cells was comparable whereas RECK protein level was very different, suggesting a post-transcriptional control. Treatment of H1299 cells with different doses of S3I-201, a STAT3 inhibitor, suppressed STAT3 activity and induced downregulation of the STAT3 target gene *cyclin D1* (Figure 1B). Interestingly, S3I-201 increased RECK protein level dose-dependently whereas no alteration of mRNA

level was found (Figure 1B). We established a stable clone from H460 cells by expressing the constitutively active STAT3 (STAT3C) gene. As shown in Figure 1C, STAT3C clone exhibited high STAT3 activity, increased cyclin D1 and reduced RECK protein. S3I-201 attenuated phospho-STAT3 and cyclin D1 whereas it restored RECK protein in STAT3C cells. Again, no change of RECK mRNA level was found. These results suggested that STAT3 may control RECK expression via a post-transcriptional mechanism.

STAT3 transcriptionally upregulates miR-92a in lung cancer cells. The *RECK* gene has been identified to be a direct target of miR-21 (Gabriely *et al*, 2008; Hu *et al*, 2008; Zhang *et al*, 2008), and miR-21 has been shown to be regulated by STAT3 in several cancer types (Loffler *et al*, 2007; Iliopoulos *et al*, 2010; Bourguignon *et al*, 2012). Therefore, it is reasonable to speculate that STAT3 may increase miR-21 to inhibit RECK in lung cancer. However, our results did not support this hypothesis. First, miR-21 level was similar in H1299 and H460 cells that exhibited very different STAT3 activity (Figure 2A). Second, STAT3C expression did not significantly upregulate miR-21 level in H460 cells

(Figure 2B). Third, S3I-201 at 50 or 100 μM almost completely inhibited phospho-STAT3 in H1299 cells, whereas it only reduced miR-21 level by 20% and 35%, respectively. We also checked the effect of S3I-201 on the expression of several miRNAs including miR-7, miR-182, miR-222, miR-372 and miR-373 that are known to target RECK mRNA. However, the expression of these miRNAs was not significantly affected by S3I-201 (Supplementary Figure 1). Hence, we searched for other potential miRNAs by bioinformatics analysis using TargetScan and miRanda databases. We found that four potential miR-92a-targeting sites were predicted at the 3′UTR of human RECK mRNA (Supplementary Figure 2). One of these sites is highly conserved in different species (Figure 3A). MiR-92a is a member of the miR-17–92 cluster that is located in the third intron of a primary transcript named C13orf25 (Ota *et al*, 2004). Expression of this cluster has recently been reported to be controlled by STAT3 in HEK293 cell and endothelial cells (Brock *et al*, 2009). We screened miR-92a expression in H1299, H460 and H358 cells and found an inverse correlation between miR-92a and STAT3 (Figure 3B). The STAT3 inhibitor S3I-201 suppressed miR-92a expression dose-dependently in H1299 cells (Figure 3C). Enforced expression of Stat3C increased miR-92a
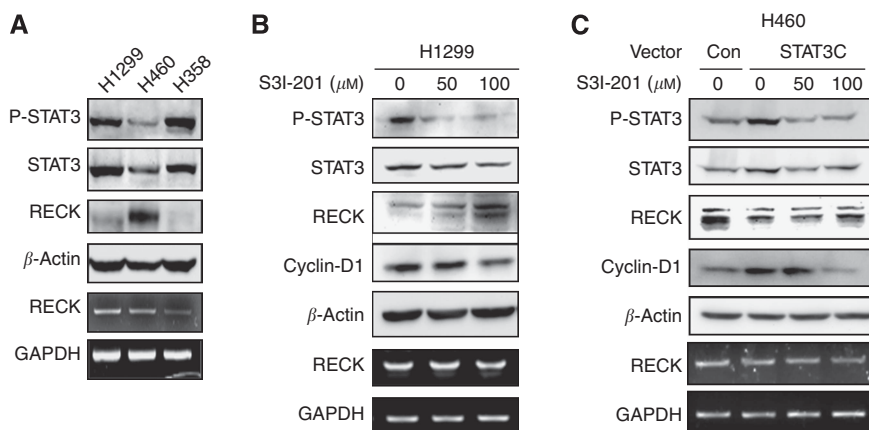

Figure 1. Signal transducer and activator of transcription 3 (STAT3) negatively regulates RECK in lung cancer cells. (**A**) The levels of RECK, STAT3 and phosphor-STAT3 were detected by western blotting in three human lung cancer cell lines. To investigate RECK mRNA expression, RT–PCR was carried out. (**B**) The STAT3-activating H1299 cells were treated with different doses of the STAT3 inhibitor S3I-201 for 48 h. Protein and mRNA levels were analysed by western blotting and RT–PCR. Cyclin D1, a transcriptional target of STAT3, was included as a positive control to verify STAT3 activation. (**C**) The H460 cells were transfected with constitutively active STAT3 expression vector to establish the stable cell line Stat3C. The Stat3C cell line was treated with different doses of S3I-201 for 48 h and protein and mRNA levels of various genes were analysed by western blotting and RT–PCR.
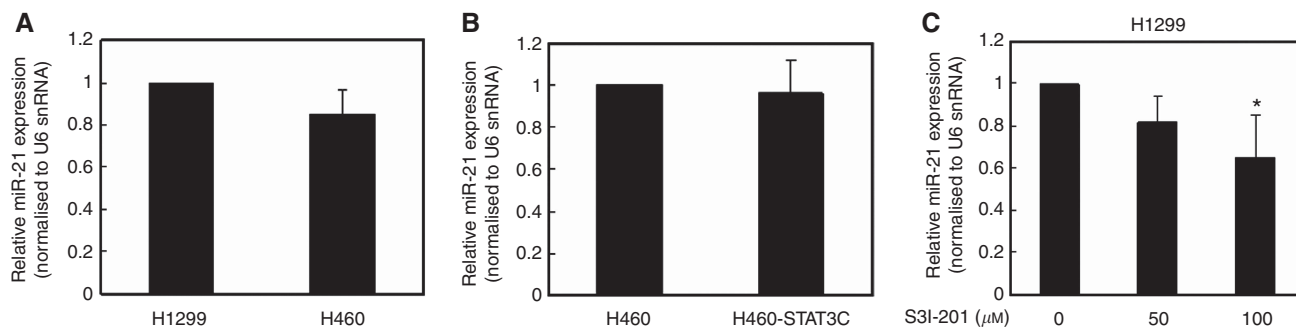

Figure 2. Signal transducer and activator of transcription 3 (STAT3) is not a key regulator of miR-21 in lung cancer cell lines. (**A**) The expression of miR-21 was detected by real-time RT–PCR and no significant difference was found in H1299 and H460 cell lines. (**B**) The level of miR-21 of H460 lung cancer cells transfected with control or constitutively active STAT3C vector was compared by real-time RT–PCR analysis. In H460 cells, STAT3C did not significantly upregulate miR-21 level. (**C**) The STAT3-activiting H1299 cells were treated with different concentrations of S3I-201 for 24 h. The expression of miR-21 was studied and the results of three independent assays were expressed as mean ± s.e. *P < 0.05 when compared with the control group.

expression in H460 cells that could be inhibited by S3I-201 (Figure 3D). An increase of microRNAs may result from enhanced transcription or altered biogenesis. Because the mature form of miR-92a was derived from C13orf25 (Figure 4A), we investigated the expression of the transcript. The C13orf25 level of H1299 cells was 1.5-fold higher than that of H460 cells (Figure 4B). S3I-201 suppressed the expression of C13orf25 in H1299 cells in a dose-dependent manner (Figure 4C). Enforced expression of STAT3C upregulated C13orf25 by 1.8-fold and S3I-201 at the concentration of 100 μM completely inhibited this upregulation (Figure 4D). Because the magnitude of change of C13orf25 level was similar to that of miR-92a, we concluded that STAT3 upregulates miR-92a mainly via increase of transcription of its host gene *C13orf25*.
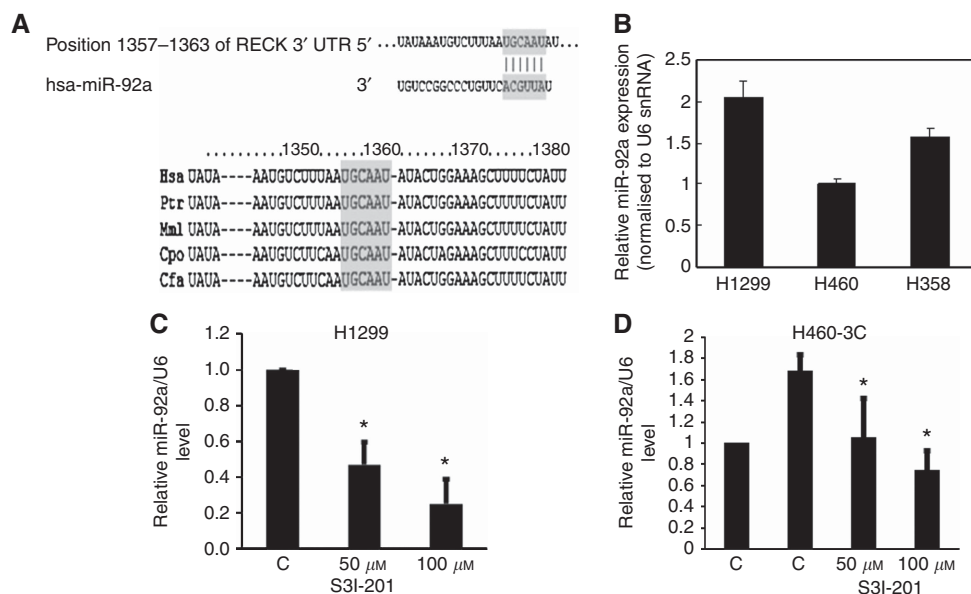

Figure 3. Signal transducer and activator of transcription 3 (STAT3) upregulates miR-92a in lung cancer cells. (A) Bioinformatics analysis identified four miR-92a binding sites in the 3′UTR of RECK mRNA and one of these sites located at 1357–1363 region is highly conserved in different species. (B) The expression of miR-92a was detected by real-time PCR in H1299, H460 and H358 cells. (C) The H1299 cells were treated with different doses of S3I-201 for 48 h and miR-92a level was analysed by real-time PCR. *$P<0.05$ when compared with the control group. (D) MiR-92a expression in H460 and H460-STAT3C cells treated with or without S3I-201 was determined by real-time PCR. *$P<0.05$ when compared with the H460-STAT3C cells treated with vehicle (0.1% DMSO).
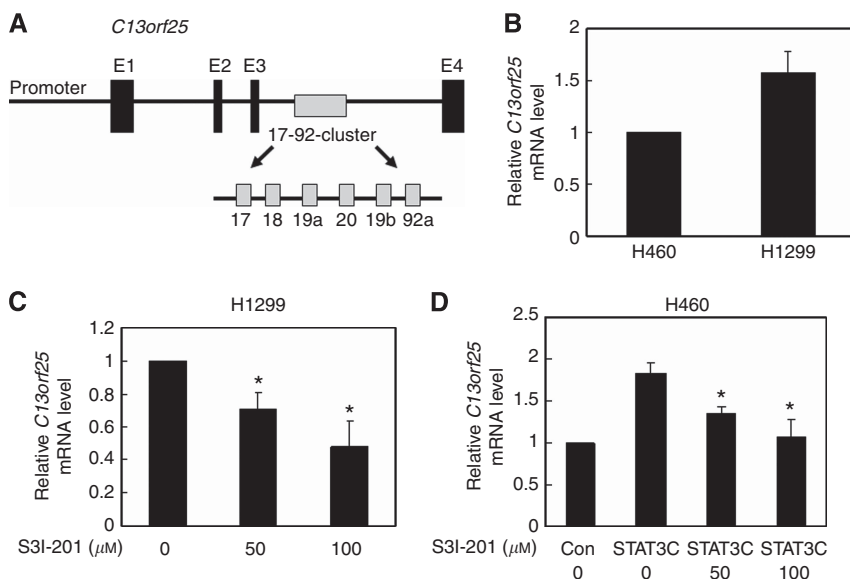

Figure 4. Signal transducer and activator of transcription 3 (STAT3) upregulates miR-92a via increase of transcription of its host gene *C13orf25*. (A) Diagram showed the structure of *C13orf25* gene and the STAT3-binding site in its promoter. MiR-17-92 cluster is located in intron 3. (B) The expression of *C13orf25* of H1299 and H460 cells was detected by real-time RT–PCR. (C) The H1299 cells were treated with different doses of S3I-201 for 48 h and C13orf25 level was analysed by real-time PCR. *$P<0.05$ when compared with the control group. (D) Expression of *C13orf25* in H460 and H460-STAT3C cells treated with or without S3I-201 was determined by real-time PCR. *$P<0.05$ when compared with the H460-STAT3C cells treated with vehicle (0.1% DMSO).
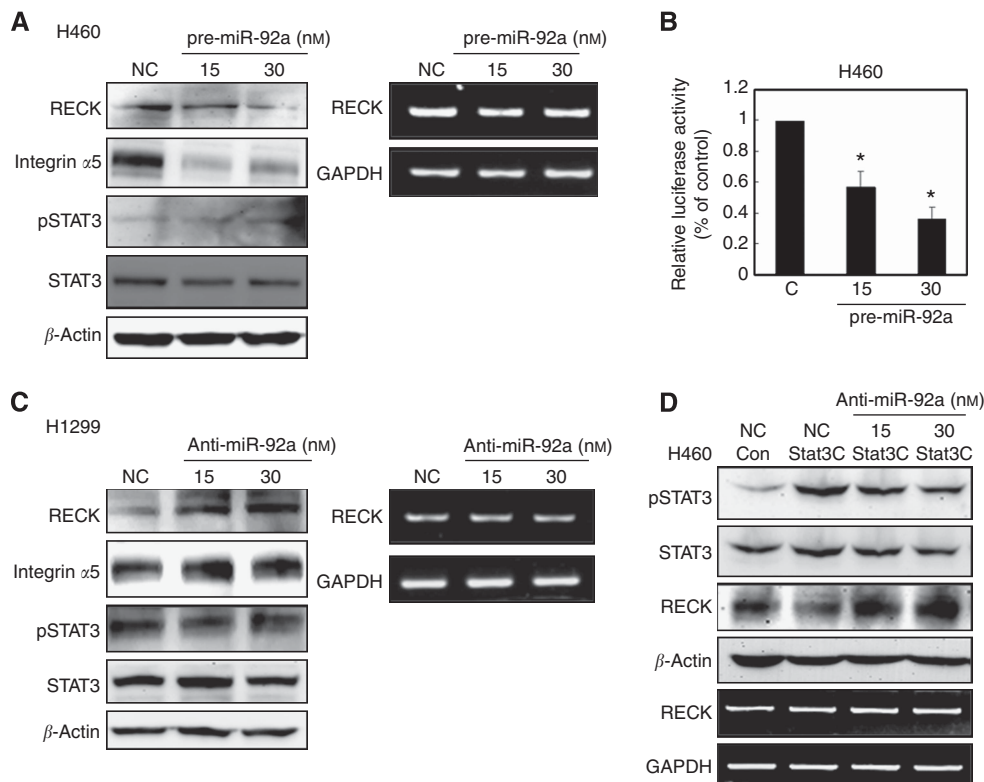
Figure 5. RECK is a direct target of miR-92a. (**A**) The H460 cells were transfected with pre-miR-92a for 72 h and the expression of RECK, integrin α5, STAT3 and phospho-STAT3 was analysed by western blotting and RT–PCR. Integrin α5 is a verified target of miR-92a and was included as a positive control. (**B**) H460 cells were co-transfected with RECK 3′UTR reporter vector and control miRNA (C, 30 nM) or pre-miR-92a (15 or 30 nM) for 24 h. Luciferase activity was determined and normalised to *Renilla* luciferase activity. Results from three independent assays were collected and the relative luciferase activity of the control group was defined as 1. *$P < 0.05$ when compared with the control group. (**C**) The H1299 cells were transfected with anti-miR-92a for 72 h and the expression of RECK, integrin α5, STAT3 and phospho-STAT3 was analysed by western blotting and RT–PCR. (**D**) The H460 and H460-STAT3C cells were transfected with control miRNA (NC, 30 nM) or anti-miR-92a (15 or 30 nM) for 72 h and the expression of STAT3 and RECK was analysed by western blotting and RT–PCR.

RECK is a direct target for miR-92a. We next addressed whether RECK is an *in vivo* target of miR92a. Delivery of pre-miR-92a into H460 cells caused a dose-dependent reduction of RECK protein (Figure 5A). No alteration of RECK mRNA was found suggesting that miR-92a inhibits RECK through translational repression. We also studied the protein level of integrin α5, an identified target of miR-92a, to verify the targeting efficacy of miR-92a in transfected cells. Indeed, integrin α5 level was attenuated (Figure 5A). Anti-miR-92a did not affect STAT3 expression or activation, suggesting that STAT3 is an upstream regulator of miR-92a (Figure 5A). When RECK 3′UTR reporter vector was co-transfected with pre-miR-92a, reporter activity was inhibited dose-dependently in H460 cells (Figure 5B). These results suggest that RECK is a direct target of miR-92a. As shown in Figure 5C, anti-miR-92a reversed RECK and integrin α5 protein level in H1299 cells. Ectopic expression of STAT3C induced down-regulation of RECK protein in H460 cells that could be reversed by co-transfection of anti-miR-92a (Figure 5D). Collectively, we conclude that STAT3 activation suppresses RECK expression by upregulating miR-92a.

MiR-92a is critical for STAT3-induced MMP activity, migration and invasion. Different functional assays were used to clarify the importance of miR-92 in the enhancement of invasiveness by STAT3. Delivery of anti-miR-92a decreased MMP-2 and MMP-9 activity as assessed by gelatin zymography in H1299 cells (Figure 6A). In accordance with the downregulation of MMP activity, cell migration and invasion were also reduced

dose-dependently. Anti-miR-92a at 30 nM inhibited the number of migrated and invade cells by ∼55% and 42%, respectively (Figure 6B). The H460 cells exhibited very low MMP-2 and MMP-9 activity (Figure 6C). Enforced expression of Stat3C in these cells dramatically increased MMP activity that was accompanied with enhanced migration and invasion (Figure 6D). All these increases were inhibited by anti-miR-92a. These results suggested that miR-92a is a critical mediator for STAT3 to promote MMP activity, invasion and migration.

DISCUSSION

The first important finding of our study is that we identify STAT3 as a key regulator that activates miR-17–92 expression in human lung cancer cells. The miR-17–92 cluster is a polycistronic miRNA gene located on the chromosome 13q31–q32 (Ota *et al*, 2004). This cluster encodes six miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a) that gathered within 800 base pair region in the intron 3 of the host gene known as *C13orf25* (Ota *et al*, 2004). Mice deficient for miR-17–92 die shortly after birth with abnormalities including lung hypoplasia, ventricular septal defect and increased B-cell apoptosis (Ventura *et al*, 2008). These data suggest the physiological importance of miR-17–92 cluster in development. Interestingly, miR-17–92 also participates in carcinogenesis. Hayashita *et al* (2005) demonstrate that miR-17–92 is markedly overexpressed in human lung cancer tissues and cell lines. Antisense inhibition of two cluster members miR-17 and
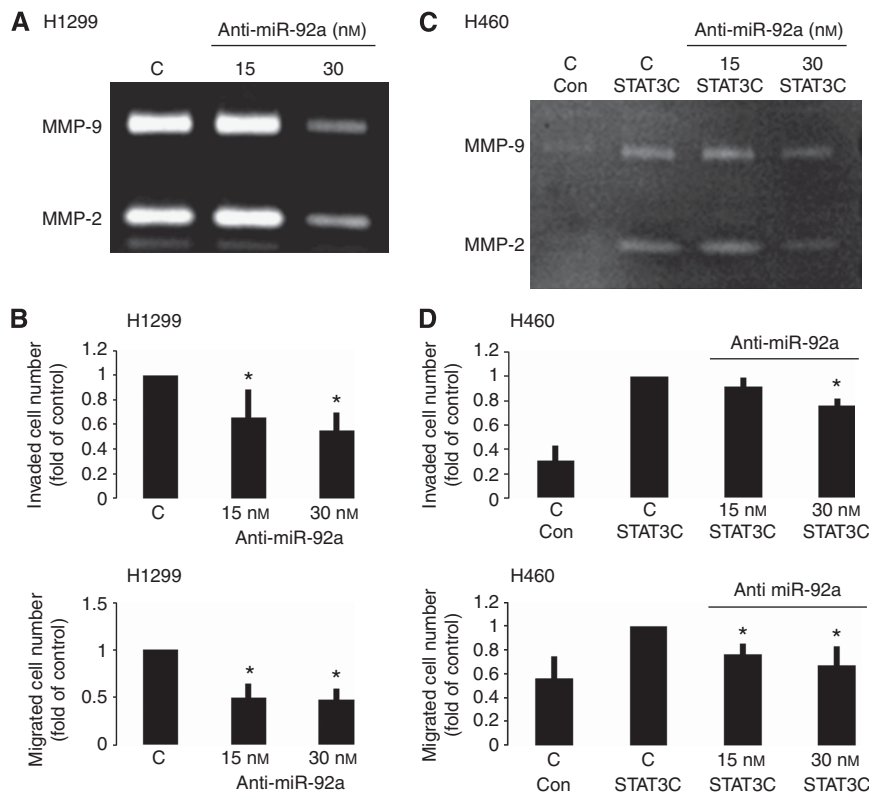
Figure 6. MiR-92a is critical for STAT3-induced MMP activity, migration and invasion in lung cancer cells. The H1299 cells were transfected with control miRNA (C, 30 nM) or anti-miR-92a (15 or 30 nM) for 72 h. The conditioned medium was harvested and subjected to zymography assay (**A**). The cells were harvested simultaneously for invasion and migration assay (**B**). *P < 0.05 when compared with the control group. (**C**) The H460 or H460-STAT3C cells were transfected with control miRNA (C, 30 nM) or anti-miR-92a (15 or 30 nM) for 72 h and the conditioned medium was harvested and subjected to zymography assay. The cell was also harvested for invasion and migration assay (**D**). *P < 0.05 when compared with the H460-STAT3C cells transfected with control miRNA.

miR-20 leads to increased apoptosis in lung cancer cells, suggesting that this cluster functions as a critical regulator in the proliferation or survival of lung cancer cells (Matsubara *et al*, 2007). Consistent with this hypothesis, forced expression of miR-17–92 under the control of surfactant protein C (a lung-specific gene) promoter causes hyperproliferation and blocks differentiation of the lung epithelium *in vivo* (Lu *et al*, 2007). However, the molecular mechanisms underlying miR-17–92 overexpression in lung cancer are still unclear. Amplification of the chromosome 13q31–32 region only occurs in a minor portion of lung cancer (Hayashita *et al*, 2005). c-Myc has been shown to activate miR-17–92 cluster in B-cell lymphoma (He *et al*, 2005a,b). However, upregulation of c-Myc is detected in <20% of lung tumours (Wu *et al*, 1998). The E2F transcription factors have also been demonstrated to activate miR-17–92 expression (Sylvestre *et al*, 2007; Woods *et al*, 2007). Interestingly, miR-17 and miR-20 inhibit E2F via a feedback loop. Whether this feedback loop plays any role in lung cancer is unclear. We demonstrate that STAT3 upregulates transcription of C13orf25 and increases expression of miR-92a in lung cancer cells. Because the magnitude of increase of miR-92a is very similar to that of C13orf25, we conclude that upregulation of miR92a by STAT3 is caused by transcriptional activation of its host gene. In ~40% of lung tumour tissues, STAT3 is activated (Cortas *et al*, 2007). Therefore, it may represent a main upstream regulator that induces miR-92a overexpression in lung cancer.

Another important finding is that we identify RECK as an *in vivo* target of miR-92a. Although the six miRNAs encoded by the miR-17–92 cluster are generally coexpressed with its host gene *C13orf25*, these miRNAs have been reported to be differentially expressed in different cancers (Venturini *et al*, 2007; Connolly *et al*, 2008; Takakura *et al*, 2008; Mi *et al*, 2010). In addition, these

miRNAs are divided into four subfamilies based on their seed sequences. MiR-17 and miR-20 have the same seed sequence (AAAGUG from nucleotides 2–7) and are the best-characterised miRNAs in this cluster. These two miRNAs exhibit oncogenic activity by targeting different tumour-suppressor genes including retinoblastoma (RB), p21, Bim and JNK2 to enhance proliferation and survival of cancer cells (Cloonan *et al*, 2008; Takakura *et al*, 2008). MiR-18a with a seed sequence of AAGGUG has been shown to regulate angiogenesis by inhibiting two antiangiogenic proteins thromospondin-1 and connective tissue growth factor (Dews *et al*, 2006). MiR-92a is the last and least described member of the miR-17–92 cluster. Overexpression of this miRNA is found in hepatocellular carcinoma, neuroblastoma and colorectal cancer (Shigoka *et al*, 2010; Nishida *et al*, 2012). However, only two target genes of miR-92a have been verified until now because this miRNA exhibits a unique seed sequence that is different from that of other cluster members. The first is Bcl-2-interacting mediator of cell death (BIM) in colorectal cancer (Tsuchida *et al*, 2011). MiR-92a was transcribed at higher levels than the other five miRNAs of the miR-17–92 cluster in colon adenoma and carcinoma. Delivery of anti-miR-92a induced apoptosis in colon cancer cell lines, suggesting an anti-apoptotic role of this miRNA. The second is DICKKOPF-3 (DKK3) in neuroblastoma (Haug *et al*, 2011). The DKK3 is a secreted protein with anticancer activity by antagonising the Wnt signaling pathway (Hoang *et al*, 2004; Lee *et al*, 2009). In neuroblastoma cell lines, N-MYC activation upregulated miR-92a expression and reduced DKK3 protein level. Investigation of neuroblastoma tissues revealed an inverse association between DKK3 mRNA and miR-92a. However, no functional assays were done to elucidate the role of DKK3 in neuroblastoma cells. In this study, we provide the first evidence that RECK is an *in vivo* target

of miR-92a. Among the miR-17-92 members, only miR-92a was predicted to target the 3′UTR of RECK mRNA. Our cell-based assay confirms the importance of miR-92a in STAT3-induced downregulation of RECK. In STAT3-activated lung cancer cells, anti-miR-92a significantly reduced migration and invasion without affecting cell viability. Therefore, the oncogenic activity of miR-92a is cell type and context dependent.

The control of invasion of lung cancer cells by the STAT3/miR-92a axis provides new strategies for treatment. Development of STAT3 inhibitors or decoys is a hot area in drug development. Several STAT3 inhibitors are in early-phase clinical trials (Debnath et al, 2012). For example, Pyrimethamine is tested for the treatment for relapsed chronic lymphocytic leukaemia and small lymphocytic lymphoma (NCT01066663, http://ClinicalTrials.gov). Another compound, OPB-31121, is also undergoing phase I/II trials for the therapy of pancreatic cancer (NCT00529113, http://ClinicalTrials.gov). It is expected that more STAT3 inhibitors will be developed for cancer treatment. Another potential strategy is siRNA therapy. Targeting of β-catenin, PLK1, Furin, RRM2, Bcl-2 and VEGF by siRNAs are tested in early-phase clinical trials (Davidson & McCray, 2011). Whether miR-92a is a rational target for cancer therapy warrants further studies. In conclusion, we demonstrate for the first time that STAT3 activation leads to miR-92a overexpression in lung cancer cells and miR-92a mediates STAT3-induced MMP activity and invasiveness by inhibiting RECK.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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