MicroRNA-7-5p mediates the signaling of hepatocyte growth factor to suppress oncogenes in the MCF-10A mammary epithelial cell

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MicroRNA-7 (miR-7) is a non-coding RNA of 23-nucleotides that has been shown to act as a tumor suppressor in various cancers including breast cancer. Although there have been copious studies on the action mechanisms of miR-7, little is known about how the miR is controlled in the mammary cell. In this study, we performed a genome-wide expression analysis in miR-7-transfected MCF-10A breast cell line to explore the upstream regulators of miR-7. Analysis of the dysregulated target gene pool predicted hepatocyte growth factor (HGF) as the most plausible upstream regulator of miR-7. MiR-7 was upregulated in MCF-10A cells by HGF, and subsequently downregulated upon treatment with siRNA against HGF. However, the expression of HGF did not significantly change through either an upregulation or downregulation of miR-7 expression, suggesting that HGF acts upstream of miR-7. In addition, the target genes of miR-7, such as EGFR, KLF4, FAK, PAK1 and SET8, which are all known oncogenes, were downregulated in HGF-treated MCF-10A; in contrast, knocking down HGF recovered their expression. These results indicate that miR-7 mediates the activity of HGF to suppress oncogenic proteins, which inhibits the development of normal cells, at least MCF-10A, into cancerous cells.
A few miRs have been identified to mediate the activity of HGF. One example is miR-124, which is downregulated in HGF-treated mesenchymal stem cells (MSC)\(^{18}\). MiR-124 downregulates Wnt/β-catenin signaling by targeting FZD4 and LRP6, thus suppressing the chemotactic migration of rat MSCs toward HGF. MiR-211 and miR-26b are also regulated by HGF in MSC, but are instead upregulated by HGF to activate PI3K/Akt signaling through targeting PTEN\(^{19}\). In renal cell carcinoma, miR-199a-3p inhibited HGF/c-Met signaling, which included the STAT3, mTOR and ERK1/2 pathways; however, whether miR-199a-3p itself is regulated by HGF has not yet been elucidated\(^{20}\).

Albeit the battery of experimental results that investigate the biological functions of miR-7 and HGF in cancer cells, there are presently no reported association between them. In this study, we explored the target genes of miR-7, which then identified HGF as an upstream regulator of the miR. This observation was then supported by the downregulation of miR-7 after inhibition of HGF using siRNA in MCF-10A mammary cells. In addition, the expression profile of miR-7 target genes was examined after the upregulation or downregulation of HGF.

**Results**

**MiR-7 affects genes involved in the cell cycle, cellular movement, cellular assembly and organization pathways.** The tumor suppressive activities of miR-7 act through specific target genes, a few of which have been identified thus far. To comprehensively understand the regulatory mechanism through which miR-7 functions in breast tissues, we performed a genome-wide expression analysis in a mammary epithelial cell line, MCF-10A, after overexpressing miR-7 using a mimic miR. Among the 47000 probes on the Illumina Expression BeadChip, 343 genes satisfied our criteria with \( P < 0.05 \) and \(|\text{fold change}| \geq 2\) compared to the control miR-transfected cells. An Ingenuity Pathway Analysis was conducted on the gene set, which resulted in the "cell cycle, cellular movement, cellular assembly and organization" network with the highest confidence (Fig. 1 and Table 1). Reassuringly, many genes that were previously identified as miR-7 targets appeared in the network, with an expression coincidence as previously claimed. In detail, MUC16, SLC2A5, SMAD6 and NCEH1 were downregulated by miR-7 and are known to possess oncogenic activities in breast cancer\(^{21,22}\), as well as a few other cancers\(^{23}\). Notably, "Mitotic roles of polo-like kinase (PLK)" was identified as the most significant canonical pathway (Fig. 2), which represents a highly conserved family of several serine/threonine kinases that regulate cell division, and are often overexpressed in tumors of various cancers\(^{24}\). In support of this, genes such as PAK1, FBXO5, CDK1 and KIF23, which directly or indirectly interact with PLK to regulate cell division, were observed to be dysregulated in the network (Fig. 1)\(^{25}\). In addition to the IPA, KEGG was also used for the pathway analysis, which identified "cell cycle"-related pathways as the top ones as like as the IPA (Supplementary Fig. S1).

**HGF acts as an upstream regulator of miR-7.** In order to investigate how miR-7 is modulated, we identified upstream regulators of the miR by performing an IPA’s upstream regulator/mediator analysis of the 343 deregulated genes in the pool. Among 20 putative regulators, HGF had the highest activation z-score (Table 2), with 32 genes affected when HGF was activated or inactivated (Supplementary Table S1). For example, SERPINE1, which was downregulated by miR-7 (2.5-fold decrease), is known to suppress HGF by activating the
cleavage of HGF26. For the upregulated genes, TPX2 (2.8-fold increase) plays a critical role in the chromosome segregation machinery during mitosis and suppresses tumor cell growth27.

The regulatory relationship between miR-7 and HGF was also investigated by examining the expression of HGF and miR-7 in 41 pairs of breast cancer and normal tissues. HGF showed an increased expression in cancer tissues compared to normal tissues, while miR-7 showed the opposite pattern, confirming the observations in previous studies (Fig. 3A,B). Notably, comparing the expression between the two genes revealed a high positive association in the normal tissues ($R^2 = 0.58$, $P < 0.01$) but not in the cancer tissues ($R^2 = 0.05$, $P < 0.05$) (Fig. 3C,D). When examined in a few other tissues including liver, head and neck, colon, and bladder, no remarkable association was found regardless normal or cancer tissue (Supplementary Fig. S2). These results imply that the association of HGF and miR-7 expression is stronger in normal tissues than in cancer tissues for breast and further that it is not a general phenomenon through body tissues.

HGF controls expression of MiR-7 and its target genes. Based on the results that HGF and miR-7 share many target genes in common and their expression is strongly associated in normal breast tissue, we examined the expression of miR-7 and its target genes in MCF-10A cells. First, the effect of HGF and miR-7 on cell proliferation was examined. As results, HGF alone increased the growth rate of cell, while miR-7 mimic alone decreased the growth rate. When miR-7 mimic was co-treated with HGF, it deteriorated the growth-stimulation effect of HGF (Supplementary Fig. S3). Next, we investigated the influence of HGF on the expression of miR-7 as well as its target genes, including $EAK$, $PAK1$, $EGFR$, $KLF4$, and $SET8$, which were all validated in our microarray assay, as well as in previous studies28–31. When MCF-10A cells were treated with 20 or 40 ng/ml HGF, a resulting

| Gene symbol | Accession no. | Description | Expression fold change$^a$
|-------------|---------------|-------------|------------------|
| KIFC1       | NM_002263.2   | kinesin family member C1 | 5.478773 |
| CEP55       | NM_018131.3   | centrosomal protein 55 | 4.961733 |
| BIRC5       | NM_001168.2   | baculoviral IAP repeat containing 5 | 4.600387 |
| AURKB       | NM_004217.2   | aurora kinase B | 4.471065 |
| KIF14       | NM_014875.1   | kinesin family member 14 | 4.438285 |
| CCNB2       | NM_004701.2   | cyclin B2 | 4.161032 |
| PRCI        | NM_199413.1   | protein regulator of cytokinesis 1 | 4.133515 |
| NCAFG       | NM_022346.3   | non-SMC condensin I complex subunit G | 3.857997 |
| RRM2        | NM_001034.1   | ribonucleotide reductase regulatory subunit M2 | 3.80439 |
| KIF4A       | NM_012310.3   | kinesin family member 4A | 3.478662 |
| CDC20       | NM_001255.2   | cell division cycle 20 | 3.475324 |
| CDC25C      | NM_022809.2   | cell division cycle 25C | 3.46151 |
| CDC48       | NM_018101.2   | cell division cycle associated 8 | 3.131205 |
| CDK1        | NM_001786.2   | cyclin dependent kinase 1 | 2.689246 |
| KIF2C       | NM_006845.2   | kinesin family member 2C | 2.67569 |
| KIF11       | NM_004523.2   | kinesin family member 11 | 2.620285 |
| CCNB1       | NM_031966.2   | cyclin B1 | 2.587337 |
| TRP13       | NM_004237.2   | thyroid hormone receptor interactor 13 | 2.548576 |
| FBXO5       | NM_012177.2   | F-box protein 5 | 2.448304 |
| PLK1        | NM_005930.3   | polo like kinase 1 | 2.413278 |
| FOXM1       | NM_021953.2   | forkhead box M1 | 2.409883 |
| AURKA       | NM_198436.1   | aurora kinase A | 2.226352 |
| KIF23       | NM_004856.4   | kinesin family member 23 | 2.02938 |
| KIFC1       | NM_002263.2   | kinesin family member C1 | 5.478773 |
| CEP55       | NM_018131.3   | centrosomal protein 55 | 4.961733 |
| BIRC5       | NM_001168.2   | baculoviral IAP repeat containing 5 | 4.600387 |
| AURKB       | NM_004217.2   | aurora kinase B | 4.471065 |
| KIF14       | NM_014875.1   | kinesin family member 14 | 4.438285 |
| CCNB2       | NM_004701.2   | cyclin B2 | 4.161032 |
| PRCI        | NM_199413.1   | protein regulator of cytokinesis 1 | 4.133515 |
| BCL11B      | NM_138576.2   | B cell leukemia/lymphoma 11B | −2.06768 |
| SMAD6       | NM_003585.3   | SMAD family member 6 | −2.25726 |
| MUC16       | NM_024690.2   | mucin 16, cell surface associated | −2.31952 |
| SLC2A5      | NM_003039.1   | solute carrier family member 2 | −2.56213 |
| RASSF5      | NM_182664.2   | Ras association domain family member 5 | −3.02865 |
| NCEH1       | NM_020792.3   | neutral cholesterol ester hydrolase 1 | −3.19179 |

Table 1. Genes in the top IPA network showing altered expression in MCF-10A cells due to miR-7. The values are obtained by dividing the expression level in miR-7-overexpressed MCF-10A by that in MCF-10A.
upregulation of miR-7 was observed (Fig. 4A); on the other hand, a downregulation of miR-7 was also observed when cells were treated with siRNA against HGF (Fig. 4B and Supplementary Fig. S4A). Both occasions showed a dose-dependent response.

To investigate whether the regulation of miR-7 by HGF functions in a feedback loop, we performed a pulse-chase experiment to check for HGF expression after miR-7 was either upregulated through mimic miR or downregulated through an inhibitor miR (Supplementary Fig. S4B,C). In both cases, no significant expression changes in HGF was observed, implying that there is no regulation of HGF by miR-7 (Fig. 4C,D).

Five genes that are known to be direct targets of miR-7 were then selected, and the effect of HGF on their expression was monitored by qPCR. Treatment of HGF to MCF-10A cells resulted in a downregulation in all genes (Fig. 5A). On the other hand, when HGF was inhibited by siRNA, an upregulation was observed in the

**Figure 2.** Top 10 canonical pathways of the genes significantly dysregulated by miR-7. The most significant canonical pathway is “Mitotic Roles of Polo-Like Kinase”. Pathways with positive and negative z-scores indicate that the pathways are activated and inhibited, respectively. Ratio is calculated as the number of genes that overlap with the corresponding pathway.

| Upstream regulator | Molecular type | Predicted activation state | Activation z-score | P-value of overlap |
|-------------------|----------------|----------------------------|--------------------|--------------------|
| HGF               | growth factor  | Activated                  | 4.953              | 2.25E-16           |
| RABL6             | other          | Activated                  | 4.796              | 4.91E-25           |
| CSF2              | cytokine       | Activated                  | 4.7                | 9.42E-16           |
| PTGER2            | g-protein coupled receptor | Activated | 4.459              | 1.52E-28           |
| VEGF              | group          | Activated                  | 4.31               | 1.96E-14           |
| RARA              | ligand-dependent nuclear receptor | Activated | 4.123              | 5.77E-08           |
| ESR1              | ligand-dependent nuclear receptor | Activated | 3.963              | 8.66E-10           |
| FOXM1             | transcription regulator | Activated | 3.696              | 4.82E-19           |
| E2F               | group          | Activated                  | 3.687              | 1.86E-16           |
| MITF              | transcription regulator | Activated | 3.483              | 4.47E-13           |
| BNP3L             | other          | Inhibited                  | −3.317             | 8.12E-10           |
| CDKN1A            | kinase         | Inhibited                  | −3.519             | 2.07E-33           |
| Irgm1             | other          | Inhibited                  | −3.592             | 1.24E-14           |
| KDM5B             | transcription regulator | Inhibited | −3.601             | 3.74E-08           |
| let-7             | microRNA       | Inhibited                  | −3.734             | 4.12E-15           |
| CDKN2A            | transcription regulator | Inhibited | −3.764             | 3.21E-11           |
| phorbol myristate acetate | chemical drug     | Inhibited                  | −4.448             | 2.89E-07           |
| TP53              | transcription regulator | Inhibited | −4.529             | 5.91E-26           |
| NUPR1             | transcription regulator | Inhibited | −5.488             | 6.02E-13           |
| calcitriol        | chemical drug   | Inhibited                  | −5.68              | 1.86E-25           |

Table 2. Potential upstream regulators or mediators of miR-7 predicted by target gene analysis. The status of the regulator that results in the same expression profile of the target genes of miR-7 in the miR-7-overexpressing MCF-10A.
expression of all genes except for SET8 (Fig. 5B). Taken together, these results suggest that HGF downregulates cell proliferation-related genes by upregulating miR-7, which acts to suppress the cancer progression of normal breast cells.

Discussion

This study aimed to explore upstream regulators of miR-7, a microRNA that has shown tumor suppressor functions in many cancers including breast cancer. To accomplish this, we adopted a strategy wherein potential upstream regulators or mediators of miR-7 were identified by screening for commonly affected genes by miR-7 and its regulators. The potential regulators appeared in diverse subcellular locations, i.e., ligands such as HGF and VEGF, receptors on the cell membrane such as PTGER2, signaling kinases such as CDKN1A and CDKN2A, and transcription factors such as TP53 and MITF.

One remarkable characteristic of the identified potential regulators is sharing of the PI3K/AKT, MAPK, or JNK in common en route the signaling pathway. Especially, 13 genes including VEGF32, RARA33 and ESR134 activate the signaling pathway by regulating MAPK. A previous study also identified TGF-β signaling to be responsible for miR-7 inhibition in the MCF-7 breast cancer cell line35. The regulatory pathways from ligands and/or receptors therefore become complicated, and the detailed pathway from HGF to miR-7 should be identified in a future study. A few of the potential mediators of miR-7 have already been known to affect or be affected by miR-7. MiR-7 was shown to be regulated by estrogen and to target signaling intermediates such as EGFR, IGF1R and IRS-236. KLF4, a direct target of miR-7, acts on the VEGF promoter to induce its mRNA and protein levels. This miR-7-KLF4-VEGF signaling axis contributes to the regulation of angiogenesis in human umbilical vein
endothelial cells. Upregulation of miR-7 targeted genes implicated in the TP53 pathway, such as Ak1 and p21, also led to the controlled growth of cortical neural progenitors.

HGF triggers multiple signaling pathways, including the conventional PI3K/AKT and MAPK pathways, coupled with the Hic-5-reactive oxygen species (ROS)-c-jun-N-terminal kinase (JNK) cascade. These signaling events eventually increase the expression of a group of genes such as N-cadherin, vimentin and Zeb1, which trigger metastatic changes including epithelial mesenchymal transition (EMT), enhancement of motility and the invasiveness of tumor cells. The majority of target genes activated by HGF have pro-proliferation activities, while those suppressed by HGF have anti-proliferation activities. HGF, therefore, has been generally considered as an oncogenic growth factor. HGF also has specific roles associated with cell proliferation in normal cells, even though these are less well-known than its oncogenic roles. Previous studies indicate that HGF increased mammary epithelial cell proliferation by acting through the PI3K-including mitogenic pathway, which thus induced a tubulo-ductal morphological response.

In contrast to previous studies, the four selected oncogenes, EGFR, KLF4, FAK and PAK1 were downregulated by HGF in MCF-10A cells. Because our result suggest that the downregulation of the genes are mediated via miR-7, we speculate that HGF acts as a double-edged sword depending on cellular status or cell type. This idea is supported by the upregulation of miR-7 in the HGF-treated MCF-10A cells, and by the strong association between miR-7 and HGF expression in normal mammary tissues. There have also been previous studies indicating the tumor-suppressive activity of HGF by abrogating the oncogenic effects of c-Myc during early stages of liver carcinogenesis, as well as enhancing the differentiation activity in mammary glands and hepatocytes. Therefore, during tumorigenesis, upregulated HGF would drive the expression of oncogenes, while in normal cells, miR-7 would mediate HGF to downregulate the same oncogenes. Recently, effective computational models such as PBMDA, HGIMDA, and RKNNMDA have been constructed to identify disease-related miRNA biomarkers. These bioinformatics-based approaches should help to gain further insight into the molecular mechanisms of HGF and miR-7. In addition, dynamic feedback modeling and Cancer Hallmark Network Framework could give us an insight to better understand the feedback loop of miR-7 in breast cancer.
Our genome-wide analysis revealed that a set of oncogenes was downregulated by miR-7, while many tumor suppressors were upregulated, supporting the tumor suppressive activity of miR-7. In addition, a group of genes that was not previously identified as targets of miR-7 was discovered to rank at the top of the network. For example, FAM83A (2.4-fold decrease) is an oncogene that activates CRAF/MAPK signaling and drives epithelial transformation\(^52\), while MUC16 (2.3-fold decrease) is a tumor marker that induces breast cancer cell proliferation by interacting with JAK2\(^21\). Whether these genes are directly regulated by miR-7, however, should be elucidated in a further study.

SET8, being different from other target genes of miR-7, was downregulated regardless of HGF overexpression or inhibition. This observation suggests the existence of other regulatory pathways between HGF and SET8, which are independent of miR-7. SET8 is the sole protein lysine methyltransferase to monomethylate histone 4 lysine 20 (H4K20) and its function has been implicated in normal cell cycle progression and cancer metastasis\(^53\). Recently, a study revealed that miR-502 directly targets SET8 to suppress cell proliferation and cell cycle\(^54\).

In conclusion, HGF was identified as an upstream regulator of miR-7 due to their sharing of a group of target genes that showed similar gene expression changes. These genes include oncogenes such as EGFR, KLF4, FAK and PAK1, which were downregulated by either HGF or miR-7. In addition, there seems to be no feedback regulation of HGF by miR-7. Because miR-7 acts as a tumor suppressor, the HGF/miR-7 pathway could potentially explain the tumor-suppressive effects of HGF in normal cells.

Materials and Methods

Cell culture and transfection. The normal epithelial breast cell line MCF-10A was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in MEBM basal medium (Lonza, Basel, Switzerland) supplemented with the MEGM Single Quot Kit (Lonza) and cholera toxin (List Biological Labs, Campbell, CA) under a humid environment with 5% CO\(_2\) at 37°C. MiR-7-5p mimic, control miR (miNC), miR-7-5p inhibitor, control inhibitor (miNC inhibitor), siHGF and siRNA control (siNC) were synthesized by Bioneer (Korea). All miRs, inhibitors and siRNAs were diluted in OptiMEM I Medium (Gibco, Los Angeles, CA, USA) and transiently transfected into cells at a final concentrations of 20 and 40 nM using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA).

Study subjects. Forty-one breast cancer tissues were obtained from patients who underwent surgery between 2013 and 2014 at the National Cancer Center (NCC) in Korea. All patients provided written informed consent.
consent to donate removed tissue to NCC, and samples were obtained according to protocols approved by the Research Ethics Board of NCC.

**RNA extraction and real-time qPCR.** Total RNA was harvested from miR- or siRNA-transfected cells and breast tissues using the ZR-Duet DNA/RNA MiniPrep kit (Zymo research, Irvine, CA, USA) according to the manufacturer's recommendations. In order to quantify the levels of mature miR-7, the extracted RNA was reverse transcribed to cDNA using the miScript II RT Kit (Qiagen, Valencia, CA, USA). Afterwards, quantitative RT-PCR (qPCR) was performed with the miScript SYBR Green PCR Kit (Qiagen) and miScript Primer Assays as the primers. For the quantification of protein coding gene's expression level, reverse transcription was carried out with ReverTra Ace qPCR RT Master Mix with gDNA remover (Toyobo, Japan) and PCR was performed with Kapa SYBR Fast qPCR Kit Master Mix ABI Prism (Kapa Biosystems, Inc., Wilmington, MA, USA). The reactions were assayed in triplicate on an ABI 7300 instrument (Applied Biosystems, Foster City, CA, USA). The expression of miR-7 and protein coding genes was normalized using endogenous U6 and GAPDH with the $2^{-ΔΔCt}$ calculation, respectively. The primers used for amplification of miRs and coding genes are listed in Supplementary Table S2.

**HGF treatment.** $3 \times 10^3$ MCF-10A cells in culture media were seeded in each well of a 96-well plate, and were treated with HGF dissolved in PBS to a final concentrations of 20 and 40 ng/ml. As a control, PBS was used to a final concentration of 5%. The cells were cultured for 24 hours and total RNA was isolated for the expression analysis of miR-7 and coding genes. To verify the cell activation by HGF, the cell growth rate was measured using Cell Counting Kit-8 (Dojindo, Japan) at 450 and 600 nm 2 h after incubation with 10 μl of CCK-8 reagent to a well. The absorption value at 450 nm was subtracted from the value at 600 nm for turbidity removal.

**Expression microarrays and pathway analysis.** One microgram of total RNA from miR-7- or miNC-transfected MCF-10A cells was used for the expression microarray of Illumina Human HT-12 v4 Expression BeadChip (Illumina, San Diego, CA). Among 47000 probes on the chip, probes with detection $P$-value $< 0.05$ and $|\text{fold change}| \geq 2$ were screened as significantly deregulated genes. Relevant networks and canonical pathways were generated using the Ingenuity Pathway Analysis (IPA) (Qiagen). The KEGG pathway enrichment analysis was performed by the KEGG Orthology Based Annotation System (KOBAS)\(^5\). The expression microarray data were deposited into the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) with the series accession number GSE102758.

**Statistical analysis.** Gene expression data were represented as the mean ± standard error of three independent experiments and analyzed by Student's t-test using SPSS for Windows, version 17.0 (SPSS, Chicago, IL, USA). Differences were considered statistically significant when the $P$-value is lower than 0.05. Linear regression was conducted to calculate the coefficient of determination ($R^2$) and the statistical significance of the correlation between miR-7 and HGF expression.

**References**

1. Rozomora, A. & Griffiths-Jones, S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res* 42, D68–73. [https://doi.org/10.1093/nar/gkt1181](https://doi.org/10.1093/nar/gkt1181) (2014).
2. Fabbri, M., Croce, C. M. & Calin, G. A. MicroRNAs. *Cancer J* 14, 1–6. [https://doi.org/10.1097/PPO.0b013e3181e4145e](https://doi.org/10.1097/PPO.0b013e3181e4145e) (2008).
3. Bushati, N. & Cohen, S. M. microRNA functions. *Annu Rev Cell Dev Biol* 23, 175–205. [https://doi.org/10.1146/annurev.cellbio.23.090506.123406](https://doi.org/10.1146/annurev.cellbio.23.090506.123406) (2007).
4. Kalinowski, F. C. et al. microRNA-7: a tumor suppressor miRNA with therapeutic potential. *Int J Biochem Cell Biol* 54, 312–317, [https://doi.org/10.1016/j.biocell.2014.05.040](https://doi.org/10.1016/j.biocell.2014.05.040) (2014).
5. Kong, X. et al. MicroRNA-7 inhibits epithelial-to-mesenchymal transition and metastasis of breast cancer cells via targeting FAK expression. *PLoS One* 7, e41523. [https://doi.org/10.1371/journal.pone.0041523](https://doi.org/10.1371/journal.pone.0041523) (2012).
6. Xie, J. et al. miR-7 inhibits the invasion and metastasis of gastric cancer cells by suppressing epidermal growth factor receptor expression. *Oncol Rep* 31, 1715–1722. [https://doi.org/10.3892/or.2014.3052](https://doi.org/10.3892/or.2014.3052) (2014).
7. Zhao, J. et al. Promoter mutation of tumor suppressor microRNA-7 is associated with poor prognosis of lung cancer. *Mol Clin Oncol* 3, 1329–1336, [https://doi.org/10.3892/mco.2015.648](https://doi.org/10.3892/mco.2015.648) (2015).
8. Zhao, J. et al. MicroRNA-7: a promising new target in cancer therapy. *Cancer Cell Int* 15, 103, [https://doi.org/10.1186/s12935-015-0259-0](https://doi.org/10.1186/s12935-015-0259-0) (2015).
9. Giles, K. M. et al. microRNA-7-5p inhibits melanoma cell proliferation and metastasis by suppressing RelA/NF-kappaB. *Oncotarget* 7, 31663–31680. [https://doi.org/10.18632/oncotarget.9421](https://doi.org/10.18632/oncotarget.9421) (2016).
10. Wu, W., Liu, S., Liang, Y., Zhou, Z. & Liu, X. MiR-7 inhibits progression of hepatocellular carcinoma by targeting KLF-4 and promises a novel diagnostic biomarker. *Cancer Cell Int* 17, 31, [https://doi.org/10.1186/s12935-017-0386-x](https://doi.org/10.1186/s12935-017-0386-x) (2017).
11. Hua, K. et al. MicroRNA-7 inhibits proliferation, migration and invasion of thyroid papillary cancer cells via targeting CKS2. *Int J Oncol* 49, 1531–1540. [https://doi.org/10.3892/ijo.2016.3660](https://doi.org/10.3892/ijo.2016.3660) (2016).
12. Kermorgant, S., Aparicio, T., Dessirier, V., Lewin, M. J. & Lehy, T. Hepatocyte growth factor induces colonic cancer cell invasiveness via enhanced motility and protease overproduction. Evidence for PI3 kinase and PKC involvement. *Carcinogenesis* 22, 1035–1042 (2001).
13. Yang, T., Wang, Y., Jiang, S., Liu, X. & Yu, Z. Hepatocyte growth factor-induced differentiation of bone mesenchymal stem cells toward hepatocyte-like cells occurs through nuclear factor-kappa B signaling in vitro. *Cell Biol Int* 40, 1017–1023, [https://doi.org/10.1002/cbi.10630](https://doi.org/10.1002/cbi.10630) (2016).
14. Niranjak, N. et al. HGF/SF: a potent cytokine for mammary growth, morphogenesis and development. *Development* 121, 2897–2908 (1995).
15. Liu, Y., Li, Q. & Zhu, L. Expression of the hepatocyte growth factor and c-Met in colon cancer: correlation with clinicopathological features and overall survival. *Tumori* 98, 105–112, [https://doi.org/10.1700/1053.11508](https://doi.org/10.1700/1053.11508) (2012).
16. Row, P. E., Clague, M. J. & Urbe, S. Growth factors induce differential phosphorylation profiles of the Hrs-STAM complex: a common node in signalling networks with signal-specific properties. *Biochem J* **389**, 629–636, https://doi.org/10.1042/BJ20050607 (2005).

17. Sakai, K., Aoki, S. & Matsumoto, K. Hepatocyte growth factor and Met in drug discovery. *J Biochem* **157**, 271–284, https://doi.org/10.1093/jb/mvu027 (2015).

18. Yue, Q. et al. MiR-124 suppresses the chemotactic migration of rat mesenchymal stem cells toward HGF by downregulating Wnt/ beta-catenin signaling. *Eur J Cell Biol* **95**, 342–353, https://doi.org/10.1016/j.ejcb.2016.06.005 (2016).

19. Zhu, A. et al. MiR-221 and miR-26b Regulate Chemotactic Migration of MSCs Toward HGF Through Activation of Akt and FAK. *J Cell Biol* **117**, 1370–1383, https://doi.org/10.1016/j.jcb.2015.08.018 (2016).

20. Huang, J. et al. miR-199a-3p inhibits hepatocyte growth factor/c-Met signaling in renal cancer carcinoma. *Tumour Biol* **35**, 3281–3289, https://doi.org/10.1007/s13277-014-0774-8 (2013).

21. Lakshmikanth, J. et al. MiC16 induced rapid G2/M transition via interactions with JAK2 for increased proliferation and anti-apoptosis in breast cancer cells. *OncoGene* **31**, 805–817, https://doi.org/10.1038/onc.2011.297 (2012).

22. Chan, K. K., Chan, J. Y., Chung, K. K. & Fung, K. P. Inhibition of cell proliferation in human breast tumor cells by antisense oligonucleotides against facilitative glucose transporter 5. *J Cell Biochem* **93**, 1143–1142, https://doi.org/10.1002/jcb.20270 (2004).

23. Leon, H. et al. SMAD6 contributes to patient survival in non-small cell lung cancer and its knockdown reestablishes TGF-beta homeostasis in lung cancer cells. *Cancer Res* **68**, 9686–9692, https://doi.org/10.1158/0008-5472.CAN-08-1083 (2008).

24. Juntermanns, B. et al. Polo-like kinase 3 is associated with improved overall survival in cholangiocarcinoma. *Liver Int* **35**, 2448–2457, https://doi.org/10.1111/liv.12839 (2015).

25. Donaldson, M. M., Tavares, A. A., Hagan, I. M., Nigg, E. A. & Glover, D. M. The mitotic roles of Polo-like kinase. *J Cell Sci* **114**, 2357–2358 (2001).

26. Campbell, D. B., Li, C., Sutcliffe, J. S., Persico, A. M. & Levitt, P. Genetic evidence implicating multiple genes in the MET receptor tyrosine kinase pathway in autism spectrum disorder. *Autism Res* **1**, 159–168, https://doi.org/10.1002/aur.27 (2008).

27. Pan, H. W., Su, H. H., Hsu, C. W., Huang, G. J. & Wu, T. T. Targeted TPK2 increases chromosome missegregation and suppresses tumor cell growth in human prostate cancer. *Onco Targets Ther* **10**, 3531–3543, https://doi.org/10.2147/OTT.S134891 (2017).

28. Reddy, S. D., Oshihko, K., Rayala, S. K. & Kumar, R. MicroRNA-7, a homeobox D10 target, inhibits p21-activated kinase 1 and regulates its functions. *Cell Death Dis* **6**, 8195–8200, https://doi.org/10.1038/cddis.2015.213 (2015).

29. Webster, R. J. et al. Regulation of epidermal growth factor receptor signaling in human breast cancer cells by microRNA-7. *J Biol Chem* **284**, 5731–5741, https://doi.org/10.1049/jbc:M014280200 (2009).

30. Okuda, H. et al. miR-7 suppresses brain metastasis of breast cancer stem-like cells by modulating KLK4. *Cancer Res* **73**, 1434–1444, https://doi.org/10.1158/0008-5472.CAN-12-2037 (2013).

31. Li, Q., Zhu, F. & Chen, P. miR-7 and miR-218 epigenetically control tumor suppressor genes RASSF1A and Claudin-6 by targeting KDM5B in breast cancer. *Biochem Bioph Res Commun* **424**, 28–33, https://doi.org/10.1016/j.bbrc.2012.06.028 (2012).

32. Wang, W. et al. MicroRNA-497 suppresses angiogenesis by targeting vascular endothelial growth factor A through the PI3K/AKT and MAPK/ERK pathways in ovarian cancer. *Oncol Rep* **32**, 2127–2133, https://doi.org/10.3892/or.2014.4349 (2014).

33. Xiao, C. et al. NLS-RARalpha Inhibits the Effects of All-trans Retinoic Acid on NB4 Cells by Interacting with P38alpha MAPK. *Int J Mol Med* **31**, 611–619, https://doi.org/10.3892/ijmm.2015.13574 (2016).

34. Lucas, T. F. et al. 17beta-estradiol induces the translocation of the estrogen receptors ER1 and ER2 to the cell membrane, MAPK3/1 phosphorylation and cultured proliferation of immature rat Sertoli cells. *Biol Reprod* **78**, 101–114, https://doi.org/10.1095/biolreprod.107.063909 (2008).

35. Akalay, I. et al. Targeting WNT1-inducible signaling pathway protein 2 alters breast human cancer cell susceptibility to specific lysis through regulation of KLF-4 and miR-7 expression. *Oncogene* **34**, 2261–2271, https://doi.org/10.1038/onc.2014.151 (2015).

36. Cochrane, D. R. et al. MicroRNAs link estrogen receptor alpha status and Dicer levels in breast cancer. *Horm Cancer* **1**, 306–319, https://doi.org/10.1016/j.sci2012.05-004-1350 (2010).

37. Li, Y. et al. Inhibition of miR-7 promotes angiogenesis in human umbilical vein endothelial cells by upregulating VEGF via KLF4. *Oncol Rep* **36**, 1569–1575, https://doi.org/10.3892/or.2016.4912 (2016).

38. Pollock, A., Bian, S., Zhang, C., Chen, Z. & Sun, T. Growth of the developing cerebral cortex is controlled by microRNA-7 through the p53 pathway. *Cell Rep* **7**, 1184–1196, https://doi.org/10.1016/j.celrep.2014.04.003 (2014).

39. Ozaki, M., Haga, S., Zhang, H. Q., Irani, K. & Suzuki, S. Inhibition of hypoxia/reoxygenation-induced oxidative stress in HGF-stimulated anti-apoptotic signaling: role of PI3-K and Akt kinase upon rac1. *Cell Death Differ* **10**, 508–515, https://doi.org/10.1038/sj.cdd.4401722 (2003).

40. Matsumoto, R. et al. Adaptor protein CRK induces epithelial-mesenchymal transition and metastasis of bladder cancer cells through HGF/c-Met feedback loop. *Cancer Sci* **106**, 709–717, https://doi.org/10.1111/cas.12662 (2015).

41. Johnson, M., Kochhar, K., Nakamura, T. & Iyer, A. Hepatocyte growth factor-induced signal transduction in two normal mouse hepatocytes. *Exp Hematol* **31**, 609–617, https://doi.org/10.1016/j.exphem.2013.03.002 (2013).

42. Santoni-Rugiu, E. et al. Kruppel-like factor 4 is involved in cell scattering induced by hepatocyte growth factor. *J Cell Sci* **125**, 4853–4864, https://doi.org/10.1242/jcs.108910 (2012).

43. Bevilacqua, L. & Kramer, R. H. HGF induces FAK activation and integrin-mediated adhesion in MTLn3 breast cancer cells. *Int J Cancer* **83**, 640–649 (1999).

44. Ro, T. B. et al. HGF and IGFr-1 synergize with SDF-1alpha in promoting migration of myeloma cells by cooperative activation of p21-activated kinase. *Exp Hematol* **41**, 646–655, https://doi.org/10.1016/j.exphem.2013.03.002 (2013).

45. Cipriano, R. et al. Conserved oncogenic behavior of the FAM83 family regulates MAPK signaling in human cancer. *Mol Cancer Res* **12**, 1156–1165, https://doi.org/10.1158/1541-7786.MCR-13-0289 (2014).

46. Houston, S. et al. Catalytic function of the PR-Set7 histone H4 lysine 20 monomethyltransferase is essential for mitotic entry and genomic stability. *J Biol Chem* **283**, 19478–19488, https://doi.org/10.1074/jbc.M705792200 (2008).
Acknowledgements
This study was supported by the Basic Science Research Program (2016R1D1A1B01009235) through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science, and Technology. Dr. H. S. Kang was supported by a grant provided by the National Cancer Center, Korea.

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Conceived and designed the experiments: S.J.K. Performed the experiments: D.J., J.H., S.P., S.L. and H.L. Analyzed the data: H.S.K. and S.J.K. Wrote the paper: D.J., S.L. and S.J.K.

Additional Information
Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-017-15846-z.

Competing Interests: The authors declare that they have no competing interests.

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