miR-205-5p-mediated downregulation of ErbB/HER receptors in breast cancer stem cells results in targeted therapy resistance

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The ErbB tyrosine kinase receptor family has been shown to have an important role in tumorigenesis, and the expression of its receptor members is frequently deregulated in many types of solid tumors. Various drugs targeting these receptors have been approved for cancer treatment. Particularly, in breast cancer, anti-Her2/EGFR molecules represent the standard therapy for Her2-positive malignancies. However, in a number of cases, the tumor relapses or progresses thus suggesting that not all cancer cells have been targeted. One possibility is that a subset of cells capable of regenerating the tumor, such as cancer stem cells (CSCs), may not respond to these therapeutic agents. Accumulating evidences indicate that miR-205-5p is significantly downregulated in breast tumors compared with normal breast tissue and acts as a tumor suppressor directly targeting oncogenes such as Zeb1 and ErbB3. In this study, we report that miR-205-5p is highly expressed in BCSCs and represses directly ERBB2 and indirectly EGFR leading to resistance to targeted therapy. Furthermore, we show that miR-205-5p directly regulates the expression of p63 which is in turn involved in the EGFR expression suggesting a miR-205/p63/EGFR regulation.

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Breast cancer is the most frequent type of cancer in women and despite the great improvement in diagnosis and treatment, relevant number of patients eventually relapses (SEER Cancer Statistics Review, 1975–2007, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2007/, based on November 2009 SEER data submission, posted to the SEER web site, 2010). Recent studies have provided strong support for the cancer stem cell (CSC) hypothesis which holds that breast cancers are driven by a subpopulation of cells within the tumor which display stem cell properties.1 These properties include self-renewal, which generates other CSCs and differentiation, which generates populations of cells forming the bulk of the tumor. There is increasing evidence that CSCs are relatively quiescent cells, resistant to chemotherapy and radiation therapy and can therefore contribute to treatment resistance and relapse. It is therefore possible that relapses observed in ErbB2-positive breast cancer patients receiving adjuvant Trastuzumab (humanized antibody anti-Her2-Heceptin) or Lapatinib (small tyrosine kinases inhibitor molecule),2,3 is due to the presence of CSCs that escape these therapeutic agents. Various mechanisms have been reported to cause resistance to targeted therapy, such as reduced ErbB2 expression, increased pro-survival signaling through alternative tyrosine kinases receptors or altered intracellular signaling leading to cellular over-proliferation.4,5

Virtually all human genes are targeted by miRNAs,6 a class of non-coding endogenous small RNAs, which modulate the expression of their target genes through base pairing with the 3’ untranslated sequence (3’-UTR) of their target mRNAs.7,8 MiRNA deregulation is widely described in cancer and has an important role in tumorigenesis.9,10 MiR-205-5p is a highly conserved miRNA, expressed in stratified squamous epithelial-derived tissues11 and in mammary gland progenitor.12 It has been shown that miR-205-5p is downregulated in breast cancer and can specifically suppress ErbB3 expression.13 Moreover, miR-205-5p has been reported to mediate the epithelial to mesenchymal transition by targeting ZEB1 and ZEB2,14,15 and it has a role in targeting several regulators of proliferation16,17 suggesting its involvement in cellular differentiation, migration and proliferation. In addition, it has been reported that miR-205-5p is regulated by p63, a p53 family member resulting in epithelial to mesenchymal transition inhibition,18 whereas the loss of the p63/miR-205 axis enhances cell migration and metastasis in prostate cancer cells.19

The TP63 gene contains two promoters that produce two proteins: the full-length TAp63 that contains functional N-terminal transcriptional transactivation (TA) domains and the ΔNp63 protein, which lacks TA domains.20 p63 has central roles in epithelial development and despite the two isoforms share some common features,20 TAp63 mainly acts as tumor suppressor and ΔNp63 as an oncogene.21,22

Here, we show that miR-205-5p is upregulated in patient-derived breast CSCs (BCSCs), compared with more
differentiated tumor cells. More importantly, we show that miR-205-5p controls CSC phenotype targeting ErbB2, p63 and EGFR, contributing to targeted therapy resistance.

Results

BCSCs show low levels of ERBB2 and EGFR. We characterized three patient-derived BCSC lines (BCSC #1, BCSC #2, BCSC #3) from three ErbB2-positive primary tumors. Immunohistochemistry analysis (Figure 1a) of the primary tumor confirms ErbB2 positivity in all three tumors, whereas CSCs derived from the tumors stain negative. Interestingly, when cells are grown as differentiating sphere-derived adherent cells (SDACs) for 14 days, they begin to show a faint positive staining. Western blot analysis confirms that spheroids retain very low expression levels of both ErbB2 and EGFR receptors that significantly increase when cells were grown as SDACs (Figure 1b). FACS analysis (Supplementary Figure S1) further confirms that indeed all three cell lines express variable (but generally low) levels of ErbB2 that again increase when cells are grown as SDACS. Interestingly, there is little correspondence between mRNA (Figure 1c) and protein levels for ErbB2 and almost none for EGFR, suggesting that expression changes in BCSCs and SDACs are at least in part mediated by non-transcriptional mechanisms. These data suggest that although CSCs show low expression levels of ErbB2 and EGFR, these receptors increase in tumor cells when they acquire a more differentiated phenotype.

BCSCs are resistant to Lapatinib. We then tested the sensitivity of cell lines grown as mammospheres to Lapatinib, an ATP-competitive reversible small-molecule inhibitor of the ErbB2 and EGFR tyrosine kinases currently used in clinics as therapy for Her2-overexpressing metastatic breast cancer patients. We confirmed a higher expression of miR-205-5p in BCSCs as compared with SDACs in all three cell lines (Figure 2a). This inversely correlates with expression of ErbB2 and EGFR, thus suggesting that miR-205 is potentially a regulator of these receptors.

miR-205-5p regulates ERBB2 and EGFR expression. To investigate whether miR-205-5p is indeed capable of regulating ErbB2 and EGFR expression, we silenced it in BCSC by cloning the miR-205-5p mature sequence in a pSIH-H1 shRNA expression lentivirus. As shown in Figure 2b, miR-205-5p knockdown results in a significant EGFR and ErbB2 upregulation at protein levels as well as at mRNA levels (Figure 2c). ZEB-1, a well-established miR-205-5p target, was used as a control to confirm functional miR-205-5p silencing.

In addition, overexpression of miR-205-5p in BCSC #1 cells by infection with pCMV-RFP-2A-puro lentivector results in strongly reduced protein levels (Figure 2d) and in reduced mRNA levels (Figure 2e) of both ErbB2 and EGFR. Similar results were obtained using the same constructs in the other two BCSC lines (data not shown), suggesting a possible direct regulation of these two genes by miR-205-5p.

Because the main algorithms for miRNA target prediction fail to find the EGFR and ErbB2 as target of miR-205, we performed an in silico analysis using the RNAhybrid algorithm (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/submission.html) to identify putative target sites for miR-205-5p in the 3′-UTR of human ErbB2 (NM_004448) (Figure 2f) and of human EGFR (Figure 2g) (NM_005228). We found few putative binding region, therefore we cloned both ErbB2 and EGFR 3′-UTR containing the predicted miR-205-5p binding site into the pGL3 control vector downstream of the luciferase open reading frame. Co-transfection of mimic premiR-205 and the WT ErbB2 3′-UTR construct in SKBR3 cells results in a significant inhibition of the luciferase activity compared with cells in which the WT ErbB2 3′-UTR construct was co-transfected with a control vector (Figure 2f). Mutation of miR-205-5p binding site within the ErbB2 3′-UTR (ERB2-3′-UTR Mut) abolishes the ability of miR-205-5p to regulate the luciferase expression resulting in the increase of luciferase activity (Figure 2f). In contrast, different results were obtained co-transfecting miR-205-5p and the WT EGFR 3′-UTR construct in SKBR3 cells. Indeed, as shown in Figure 2g, miR-205-5p was not able to decrease luciferase activity, even at longer time points (24, 48 and 72 h after transfection). These data suggest that miR-205-5p might modulate EGFR expression indirectly, by targeting other key factors involved in EGFR regulation.

miR-205-5p controls p63 expression. To further investigate the indirect regulation of EGFR expression by miR-205-5p,
Figure 1 ERBB2 and EGFR expression pattern in BCSCs and during differentiation. (a) Immunohistochemistry of three different paraffin-embedded primary tumor tissues (left), CSC lines derived from tumors (centre) and SDACs (right) stained with anti-HER2 antibodies (pink-brown) (Scale bar: 20 μM). ERBB2 is expressed in primary tumors but not in CSCs lines and starts to be re-expressed under differentiating condition (SDAC). (b) Western blot analysis of BCSC lines (BCSC#1, BCSC#2 and BCSC#3) and stem cells under differentiating condition (SDAC) for 3.5 and 7 days using antibodies against ERBB2, EGFR and Actin as a loading control. The corresponding molecular weights are indicated on the left (KDa). ERBB2 and EGFR expression increases during differentiating condition. (c) qRT-PCR of ERBB2 and EGFR levels of BCSC lines (BCSC#1, BCSC#2 and BCSC#3) and SDAC of the same lines differentiated for 3.5 and 7 days. Data represent mean ± S.D. of three different experiments analyzed in triplicate. Statistical significance was analyzed using Student's T test (*P<0.05). (d) BCSCs are resistant to Lapatinib treatment. Cell proliferation assay of BCSCs and SDAC untreated (ctr) or treated with 0.5 μM of Lapatinib at the indicated time points (days). SDAC were differentiated for 3.5 days and then plated for growth curve analysis. BCSCs express low receptors levels and are more resistant to treatment than SDAC. Data represent mean ± S.D. of three different experiments and P values are shown in the graphs.
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we studied the role of p63 in this pathway, because it has been reported to promote the transcription of EGFR. Interestingly, it has also been shown that p63 controls miR-205-5p expression. To evaluate the potential role of p63 in miR-205-5p-ERBB family axis, we first checked the expression levels of p63 in our model. As shown in Figure 3, all three lines tested express detectable mRNA (Figure 3a) and protein (Figure 3b) levels of p63. In addition, we tested whether p63 was also able to drive the expression of EGFR in BCSCs as previously reported. As shown in Figure 3c, silencing of p63 in BCSC #1 cells results in reduced expression of EGFR, whereas upregulation of the ΔN but not the TA isoform results in increased EGFR levels (Figure 3d). Interestingly, p63 levels increase in more differentiated cancer cells as compared with cells grown as spheroids in parallel with the reduction of miR-205-5p expression (Figure 3e). We therefore investigated whether miR-205-5p is capable of regulating p63 expression. As shown in Figure 4a, miR-205-5p knockdown results in a significant p63 upregulation both at the mRNA and protein levels. Consistently, miR-205-5p overexpression results in reduced p63 levels (Figure 4b). Bio-informatic analysis of p63 3′-UTR identified a putative miR-205-5p seed region (Figure 4c, left). We therefore cloned the p63 3′-UTR containing the miR-205-5p binding site into the pGL3 control vector downstream the luciferase gene. Co-transfection of premiR-205 and the WT p63 3′-UTR construct in SKBR3 cells resulted in significant inhibition of luciferase activity compared with the cells in which the WT p63 3′-UTR construct was co-transfected with a control vector (Figure 4c, right). These data were confirmed by mutation of miR-205-5p binding site within the p63 3′-UTR (p63-3′-UTR Mut) that abolishes the ability of miR-205-5p to regulate the luciferase expression leading to an increase of luciferase activity. We therefore believe that miR-205-5p regulates expression of EGFR through regulation of ΔNp63. Interestingly, we confirm that as reported in literature, p63 regulates miR-205-5p expression thus creating a regulatory feedback loop. In fact, p63 silencing...
results in reduced miR-205-5p levels (Figure 4d), whereas upregulation of the \( \Delta N \) but not the TA isoform results in significant increase of miR-205-5p levels (Figure 4e).

We therefore sought out to re-sensitize BCSCs to Lapatinib treatment downregulating mir-205-5p, that we showed is responsible for reduced expression of EGFR and ErbB2 in these cells. As shown in Figure 4f, silencing miR-205-5p in BCSCs and treating them with Lapatinib strongly reduces cell proliferation, sensitizing cells to anti-Her2/EGFR treatments. These findings indicate that miR-205-5p is able to regulate ErbB receptors expression thus leading to targeted therapy resistance of BCSCs.

**Discussion**

Growing evidence suggest that a subset of cells within the tumor, referred as CSCs are capable of escaping anti-cancer treatment driving tumor progression, metastasis and relapse.\(^1\) Many studies have therefore focused on the identification of pathways that are essential in determining the CSC phenotype. It is well known that miRNAs contribute to carcinogenesis and tumor development acting as oncogenes or as tumor suppressors depending on specific targets and tumor microenvironment. Altered miR-205-5p expression has been involved in several types of solid tumors and, to date, its target include tumor suppressors like PTEN\(^12\) and SHIP2,\(^31\) oncogenes such as HER3,\(^32\) PKC\(\varepsilon,\)\(^17\) pro-metastatic factors Zeb1 and Zeb-2,\(^14\) and the angiogenic gene VEGFA.\(^32\) Moreover, miR-205-5p has been shown to be essential for mouse development,\(^33,11\) particularly for the expansion of progenitor and stem cell populations in epidermis, hair follicles and more importantly in mammary gland during neonatal development.\(^33,12\) therefore, we hypothesize its mis-regulation could be translated in maintaining the CSC phenotype. In breast cancer, miR-205-5p was found to be either up- or downregulated compared with normal tissue, but its

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**Figure 4** miR-205-5p regulates p63 expression in BCSCs. (a) miR-205-5p regulates p63 levels. qRT-PCR (left) and western blotting analysis (right) of p63 expression levels in BCSC#1 infected with miR-205-5p silencing lentivirus (shmiR-205-5p). (b) qRT-PCR (left) and western blotting (right) of p63 expression levels in BCSC#1 infected with PremiR-205 lentivirus. miR-205-5p overexpression results in p63 downregulation mainly at protein levels. (c) miR-205-5p directly targets p63-3'-UTR and alignment of the seed sequence with both WT and mutated p63 3'-UTR. On the right, SKBR-3 cells were co-transfected for 48 h with pGL3-p63 3'-UTR luciferase construct (WT or Mut 3'-UTR), premiR-205 construct or a control vector (CTR). Cloning p63 3'-UTR WT, and not the mutated one, into a luciferase reporter gene leads to diminished luciferase activity in the presence of PremiR-205. (d) p63 regulates miR-205-5p expression. qRT-PCR of miR-205-5p expression levels in BCSC#1 infected with shp63 lentivector (shp63) or a control vector (plentilox). (e) qRT-PCR of miR-205-5p expression levels upon T ap63 or \( \Delta N p63 \) overexpression in BCSC#1. \( \Delta N p63 \) overexpression results in miR-205-5p upregulation. (f) miR-205-5p downregulation re-sensitizes BCSCs to Lapatinib treatment. Percentage of cell growth of BCSC#1, BCSC#2 and BCSC#3 infected with shmiR-205-5p lentivector (shmiR-205-5p) or a control vector (CTR) and treated or untreated with 0.5 \( \mu M \) Lapatinib for 6 days.
expression in BCSCs remains still unknown. Here, we show that \( \text{mir-205-5p} \) is highly expressed in human BCSCs compared with more differentiated tumor cells and that it directly targets ERBB receptors leading to their downregulation. In fact while it was known that ERBB3 is a direct target of \( \text{mir-205-5p} \), we show that it also regulates ErbB2 and EGFR. While ErbB2 appears to be a direct target of this miR, EGFR regulation is mediated through the regulation of p63 that has already been shown to be able of regulating transcription of EGFR.\(^{30}\) Indeed, here we show for the first time that p63 is a direct target of \( \text{mir-205-5p} \) and confirm previous reports showing that in turn \( \text{ΔNp63} \) regulates \( \text{mir-205-5p} \) expression.\(^{19,18}\) These data show that, therefore, there is a feedback loop finely regulating expression of \( \text{mir-205-5p} \) and p63 that have a role in determining some of the phenotypic features of BCSCs including surface expression of ERBB receptors.

Intriguingly, we show that low expression of ERBB receptor family members in BCSCs contributes to resistance of these cells to agents such as Lapatinib used in breast cancer therapy. Survival of these cells could then lead to tumor progression and suggests that \( \text{mir-205-5p} \) could be an important target to improve outcome of patients with Her2-overexpressing breast cancer.

In conclusion, we want to point out that we identified for the first time two new direct targets of \( \text{mir-205-5p} \) and shown that this miRNA has an important role in determining BCSCs phenotype and contributes to their resistance to targeted therapy.

Materials and Methods

BCSC isolation and culture. BCSCs were isolated from human breast cancer tissues obtained from patients as previously described\(^{34}\) and were cultured in a selective medium\(^{34}\) supplemented with 10 ng/ml hFGF (Peprotech, London, UK), 20 ng/ml EGF (Peprotech) to a final concentration 5 × 10\(^4\) ml in ultra low attachment flask (Corning, New York, NY, USA) at 37 °C in a 5% (v/v) CO\(_2\) humidified chamber. BCSCs were induced to differentiate in order to obtain SDA cells by culturing them in adherent condition in D-MEM with high glucose (Euroclone, Milan, Italy) supplemented with 10% (v/v) fetal bovine serum (Euroclone). The tumor s were histopathologically classified as follows: BCSC1 is an invasive ductal carcinoma, grading 2, ER 90%, PR 60%, HER2/neu 3+ and ki67 > 10%; BCSC2 is an invasive ductal carcinoma, grading 2. ER 90%, PR 60%, HER2/neu 3+ and ki67 25%; BCSC4 is an invasive ductal carcinoma, grading 2. ER 80%, PR 80%, HER2/neu 3+ and ki67 > 10%. HER2 status has been assigned according to the definition of the American Society for Clinical Oncology and the College of American Pathologists.\(^{35}\) Ki67 was assessed by immunohistochemical staining with an anti-ki67 antibody (DAKO, Glostrup, Denmark).

Quantitative reverse transcription-PCR. For mRNA detection, RNA was extracted from BCSCs by using miRvana miRNA isolation kit (Ambion by Life Technologies). A total of 50 ng of RNA was used for reverse transcription using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystem by Life Technologies) with the following stem loop specific primers: \( \text{mir-205-5p RT: 5'-gcttg cgctagcggcttagctgctgcagcactcgagttc-3'} \) and \( \text{mir-205-5p R: 5'-ccctcg aggctgcctcggtaaggtagta-3'} \) for \( \text{mir-205-5p} \) expression. \( \text{ΔΔCT} \) was calculated using the following formula:

\[
\text{ΔΔCT} = \text{ΔCT}_{\text{Target}} - \text{ΔCT}_{\text{Internal control}}
\]

Infections. Lentiviruses were produced by transient cotransfection of a three-plasmid expression system in the packaging 293T cells, using the calcium phosphate transfection kit (Invitrogen, Life Technologies). Cells were incubated for 7 h with the transfection reagents and viral supernatant was collected 48 h after transfection and filtered through 0.45 μm pore vacuum sterile filtration system (Millipore, Life Science, Darmstadt, Germany). Then, BCSCs were plated in a six-well ultra-low attachment plate (Corning) with viral supernatant and 4 μg/ml of polybrene. Plates were centrifuged for 45 min at 1800 r.p.m. and incubated at 37 °C for 75 min in a 5% CO\(_2\) humidified chamber. Cells were then washed twice and replated in fresh medium.\(^{36}\) Infection efficiency was assessed by flow cytometry (FACSCanto II Instrument, BD Biosciences, San Jose, CA, USA) 48 h post-infection evaluating the percentage of GFP-positive or RFP-positive cells measured. Data were analyzed with CELLQuest software (BD Biosciences).

Real-time PCR was performed by using FastStart Universal Probe Master (Roche, Roche Diagnostics, Basel, Switzerland) and Universal Probe Library, Probe #21 (Roche) according to the following primers: \( \text{mir-205-5p Fw: 5'-gaggctgccagtgcggagctctgcagcactcgagtgtgagggtaagagttaa-3'} \) and \( \text{mir-205-5p Rw: 5'-ggccactagtgcctcacatctgagaccagggaggtgtaacctgagcagtgcagcactc-3'} \) for \( \text{mir-205-5p} \) expression. \( \text{ΔΔCT} \) was calculated using the following formula:

\[
\text{ΔΔCT} = \text{ΔCT}_{\text{Target}} - \text{ΔCT}_{\text{Internal control}}
\]
were as follows: Tqp63 (NM_003722.4) F: 5'-ttgtagtatgctagctgtta-3' and R: 5'-gttggactcctgctgta3'-5'; Tqp63 (NM_001114960.1) F: 5'-gtgctgaaactaatcttgag-3' and R: 5'-gtgctgctgatggctgcta-3'. For ErbB2 (NM_004448.2) F: 5'-ggaaactctgaacctct-3' and R: 5'-ccctccatctcgctggta-3'; for EGFR (NM_005228.3) F: 5'-ttcc tccacgctgtggta-3' and R: 5'-gctgagcaggtctgtgg-3'; for ZEB1 F: 5'-ggcacaagctcagaaaga-3' and R: 5'-ggctgctgacatagagai-3'.

All genes expression were normalized using human β-actin as housekeeping gene, and primers used were AccF 5'-cagccaaatggctgttttgg-3' and AccR 5'-aagccggccttgcacat-3'. Relative quantification of gene expression was calculated according to the comparative method of 2-ΔΔCT.

**Microarray.** Total RNA was extracted from BCSC#1 BCSCs and from BCSC#1 differentiated cells at 7 days, according to Trizol protocol (Ambion by Life Technologies). Total RNA was used for miRNA microarray analysis (G4470E, Agilent Technologies). This chip allows the simultaneous analysis of 723 human miRNAs (miBase release 10.1); RNA labeling and hybridization were performed in accordance to the manufacturer's indications, Agilent scanner and the Feature Extraction 10.5 software (Agilent Technologies) were used to obtain the microarray raw data. Microarray results were analyzed using the GeneSpring GX 12 software (Agilent Technologies). Data transformation was applied to set all the negative raw values at 1.0, followed by Quantile normalization and log2 transformation. Differentially expressed miRNAs were identified by using a moderated t-test and Benjamini-Hochberg correction (adjusted P<0.05). Differentially expressed genes were used in Cluster Analysis, using the Pearson correlation as a measure of similarity.

**Western blotting.** Proteins were extracted with a lysis buffer (TRIS-HCl 50 mM pH 8, NaCl 150 mM, Triton X-100 1%, NaF 100 mM, EDTA 1 mM, MgCl2 1 mM, Glycerol 10%) containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) and a phosphatase inhibitor cocktail (Roche) as previously described. Equal amounts of total protein were subjected to SDS-PAGE and then electrophoresed to nitrocellulose membranes. The membranes were blocked with 5% non-fat milk in PBS with 0.1% Tween 20 and incubated overnight using the following antibodies: anti-β-Actin A5441 (Sigma-Aldrich), anti-EGF Receptor (D38B1) XP Rabbit mAb (Cell Signaling, Danvers, MA, USA), anti-ErbB2 (D8F12) XP Rabbit mAb (Cell Signaling, Danvers, MA, USA), anti-ZEB1 (Millipore, Life Science), anti-p63 Y4A3 (Sigma-Aldrich) or (Cell Signaling, Danvers, MA, USA), anti-ErbB2 (D8F12) XP Rabbit mAb (Cell Signaling). After wash, membranes were hybridized with horseradish peroxidase-conjugated secondary antibodies (rabbit and mouse, Bio-Rad, Hercules, CA, USA). Detection was performed with Plus-ECL chemiluminescence kit (PerkinElmer, Inc., Waltham, MA, USA) or with SuperSignal West Dura extended duration substrate kit (Thermo Scientific, Waltham, MA, USA).

**Immunohistochemistry.** BCSCs and SDACs derived from BCSC#1, BCSC#2 and BCSC#3 lines, were spotted on microscope slides with cytospin at 900 r.p.m. for 3 min and then were fixed in formalin 10% neutral buffered for 15 min. BCSC#2 and BCSC#3 lines, were spotted on microscope slides with cytospin at 4 °C in the dark until acquisition. A FACSCanToll flow cytometer, running with FACSData software (BD Biosciences), was used for sample acquisition and analysis.

**Cell proliferation assay.** BCSCs, SDACs and BCSCs infected with shnmR-205-2p were seeded into six-well plate at 5 x 10^4 cells per well. Viable cell count was performed with Trypan Blue reagent (Sigma-Aldrich) at the indicated time points. When indicated, cells were treated with 0.5 μM Lapatinib (Biovision, Milpitas, CA, USA).

**Bioinformatics.** miR-205 target sites on p63 3'-UTR, ERBB2 3'-UTR and EGFR 3'-UTR were predicted by RNA Hybrid software available at http://rbiserv.techfak.uni-bielefeld.de/mahybrid/submission.html

**Conflict of Interest**

The authors declare no conflict of interest.

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