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Research Article

MicroRNA-100 targets IGF1R and regulates migration in a breast cancer model of epithelial-mesenchymal transition

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Abstract. Luminal breast tumors typically have a lower risk of metastasis due to the epithelial nature of the cells. Strong adhesion between epithelial cancer cells lowers their motility, migration rate, and invasive potential. Despite the nature of epithelial breast tumors, they may still undergo metastasis, which is thought to be initiated by epithelial-mesenchymal transition (EMT). EMT is a process through which epithelial cells acquire the phenotype of mesenchymal cells, which have enhanced invasive potential in cancer. MicroRNAs, small regulatory RNAs that inhibit gene expression, play a role in the regulation of EMT. This study demonstrates that microRNA-100 (miR-100) is upregulated in mesenchymal breast cancer cells (MCF-7_M) versus epithelial breast cancer cells (MCF-7) via a mammosphere-induced EMT. MiR-100 may play a role in EMT by targeting the insulin like growth factor 1 receptor (IGF1R). Inhibition of miR-100 in MCF-7_M cells significantly increased endogenous IGF1R expression. IGF1R has been implemented in the initiation of tumorigenesis; however expression of this receptor also strengthens E-cadherin mediated adhesion in epithelial cells. It is hypothesized that upregulation of miR-100 in mesenchymal breast cancer cells decreases IGF1R expression to facilitate increased motility and invasive potential, and inhibition of miR-100 in mesenchymal breast cancer cells (MCF-7_M) resulted in decreased cell migration. This study enhances the understanding of the role of microRNAs in breast cancer metastasis, and may provide novel targets or biomarkers of cancer progression.

Introduction

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer-related deaths among women (Wang and Zhou, 2013). All cancers become increasingly more lethal when they undergo metastasis, and over 90% of cancer-related deaths are due to metastasis (Sporn, 1996). Due to the nature of this process, tumors that are likely to metastasize exhibit high migration, cell motility, and proliferation rates. One clinical subtype of breast cancer, Basal-like, displays a high rate of metastasis. Patients with these tumors experience poorer prognoses due to the invasive nature of these cancers and limited treatment options. Tumors of another subtype of breast cancer, Luminal A, are less aggressive and patients have a better prognosis as a result of the epithelial phenotype of these tumors. The epithelial phenotype of Luminal A tumors contributes to the decreased incidence of metastasis due to the tightly-packed, contact-dependent growth patterns of the cells (Kittaneh

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et al., 2013; Peppercorn et al., 2008). High levels of junction proteins, such as E-cadherin, result in strong cell-to-cell and extracellular matrix adhesion, disabling cells from migrating away from the primary tumor. In addition, Luminal A type breast cancer cells are characterized as estrogen receptor (ER) positive and require estrogen to grow, so anti-estrogen therapies such as tamoxifen and aromatase inhibitors are effective in halting tumor growth. Although Luminal A tumors have a low incidence of metastasis, the potential to give rise to more aggressive phenotypes may be facilitated by epithelial-mesenchymal transition (EMT; Kittaneh et al., 2013; Peppercorn et al., 2008).

EMT allows epithelial cells to acquire the phenotype and characteristics of mesenchymal cells, which are characterized by a loss of adhesion and cytoskeletal rearrangement. EMT increases cell motility, migration, and proliferation rate, increasing the risk of metastasis and recurrence (Moody et al., 2005; Peppercorn et al., 2008; Wang and Zhou, 2013). For instance, upregulation of the EMT promoting transcription factor Snail is sufficient to promote neu-induced mammary tumor recurrence in mice (Moody et al., 2005). Since EMT is believed to be the initiating step in metastasis and recurrence, metastatic and secondary tumor cells often display a variety of mesenchymal and EMT-related molecular markers. Recent research has focused on the molecular mechanisms that regulate EMT in breast cancer (Moody et al., 2005; Guttilla et al., 2012; Wang and Zhou, 2013). Mammosphere culture was shown to be effective in inducing EMT in breast cancer cells, allowing for analysis of molecular changes associated with EMT (Guttilla et al., 2012).

Mammosphere culture is a form of cell culture that enriches for cancer stem cells (tumor-initiating cells). Previous work has shown that culturing MCF-7 luminal epithelial breast cancer cells as mammospheres resulted in a stable EMT, as confirmed by molecular profiling (Guttilla et al., 2012). The resulting cells displayed characteristics of mesenchymal cells, and the new cell population was termed MCF-7_M. Cancer-related microRNAs were examined prior to and following mammosphere-induced EMT. MicroRNAs are short, 20-24 nucleotide RNA molecules that repress gene expression by binding to the 3′ untranslated region (3′ UTR) of their target mRNA, which leads to translational inhibition or degradation of the mRNA transcript (Farazi et al., 2013). It has been well established that microRNAs have a large impact on the nature of cancer cells, and aberrant microRNA signaling is linked to metastasis, angiogenesis, enhanced survival, proliferation, and EMT in a variety of cancers (Guttilla et al., 2012; Iorio and Croce, 2009). In this study, microRNA-100 (miR-100) was found to be upregulated in MCF-7_M cells, suggesting this microRNA may play a role in EMT (Guttilla et al., 2012). To understand the potential role of miR-100 in EMT, bioinformatics databases (TarBase), as well as recent studies, have identified insulin-like growth factor 1 receptor (IGF1R) as a target of miR-100 (Doghman et al., 2010; McKinsey et al., 2011).

IGF1R is a receptor tyrosine kinase that promotes tumorigenesis when activated by its ligands, IGF-1 and IGF-2. IGF1R is upregulated during tumor formation to enhance cell growth, survival, and evasion of apoptosis by activating the PI3K/Akt pathway (Sachdev and Yee, 2001). Although IGF1R expression has tumor promoting capabilities, IGF1R also increases E-cadherin facilitated cell adhesion in MCF-7 cells. IGF1R overexpression strengthens cell adhesion by upregulating zonula occludins-1 (ZO-1), a junction protein of the E-cadherin complex. Therefore, repressed levels of IGF1R are associated with loss of cell adhesion, increased cell motility, and overall greater metastatic potential (Mauro et al., 2001; Pennisi et al., 2002). It is hypothesized that tumors may utilize IGF1R to begin the formation of a tumor, but then downregulate IGF1R via miR-100 to undergo EMT.

Inhibition of endogenous miR-100 in MCF-7_M cells was utilized to investigate the potential interaction between miR-100 and IGF1R in breast cancer cells, and elucidate the role of miR-100 in EMT. The expression of miR-100

was significantly upregulated in MCF-7_M cells compared to MCF-7 cells, and the expression of IGF1R was inversely correlated with the expression of miR-100. It was confirmed that endogenous IGF1R is a target of miR-100 in breast cancer cells. Functional assays performed in MCF-7_M cells demonstrated that inhibition of miR-100 decreased cell migration. Confirming IGF1R as a target of miR-100 in breast cancer cells and understanding the role of these molecules in EMT provides insight into the molecular changes involved in cancer progression.

Materials and Methods

Cell culture

MCF-7 and MCF-7_M breast cancer cell lines were cultured in media containing DMEM/F12, 10% fetal bovine serum, 1% pyruvate, 1% glutamine, and 1% penicillin/streptomycin mixture. Cells were maintained at a temperature of 37°C and 5% CO_2 on adherent cell culture plates. Media was changed when necessary and cells were passaged when confluent through trypsinization.

RNA isolation from MCF-7 and MCF-7_M cell

RNA was purified using Trizol® Reagent (Life Technologies) according to manufacturer's protocol, and pellets were resuspended in nuclease-free water. RNA samples were quantitated using a Qubit fluorometer (Invitrogen).

cDNA synthesis and PCR for microRNA detection

For cDNA synthesis of microRNAs, a stem-loop gene-specific primer was used for reverse transcription as previously described (Guttilla et al., 2012). Briefly, 1 μg of total RNA, 2 pmol gene specific primer, and 0.5 mM of dNTPs were incubated at 65°C for 5 min. Superscript™ III Reverse Transcriptase (100 units, Invitrogen) and kit reagents were added to a final volume 20 μL and incubated at 55°C for 60 min. The enzyme was denatured 70°C for 15 min. PCR was performed with 0.2 μM primers, and 4 μL cDNA in 50 μL, using 95°C/5 min; 95°C/30 sec, 55°C/30 sec, 72°C/30 sec for 30 cycles; 72°C/10 min. Products were resolved on 10% non-denaturing acrylamide gels, stained with Sybr Gold and imaged. All assays were performed in triplicate using independent RNA samples.

cDNA and endpoint PCR for target gene detection

cDNA was synthesized from 1 μg total RNA using iScript Reverse Transcription Supermix (Bio-Rad) according to the manufacturer's protocol. Standard PCR was performed with 0.2 μM primers, and 2 μL cDNA in 50 μL, using 95°C/5 min; 95°C/30 sec, 52°C/30 sec, 72°C/30 sec for 30 cycles; 72°C/10 min. 2 μL cDNA and 10μM gene specific primers were used for standard PCR protocol. PCR products were resolved on a 2% agarose gel and stained with ethidium bromide. All assays were performed in triplicate using independent RNA samples. Primers were designed using the Primer 3 program.

Western blot

Whole cell protein lysates were isolated from the indicated cell lines using RIPA lysis buffer supplemented with 1X protease inhibitor cocktail set I (Millipore). Protein samples were quantitated using a Qubit fluorometer (Invitrogen). Samples were prepared in 5X SDS Loading Buffer supplemented with 5% β-mercaptoethanol. 10% running and 4% stacking SDS-PAGE gels were used to run protein samples in 1X Tris-Glycine-SDS running buffer. Protein was transferred to nitrocellulose membranes using standard protocols. Membranes were blocked in 5% milk in PBST and incubated overnight in 1:250 α-IGF1R and 1:100 α-actin diluted primary antibodies in blocking solution (Santa Cruz Biotechnologies). Membranes were incubated in 1:500 AP-conjugated anti-mouse secondary antibody in blocking solution. Blots were developed using a NBT/BCIP solution. All assays were performed in triplicate using independent protein samples.

Antisense RNA treatments

MCF-7_M cells were seeded in 6-well plates at 3.5×10^5 cells per well. Cells were transfected using Lipofectamine® RNAiMAX Reagent (Life Technologies) according to manufacturer's protocol using OptiMEM reduced serum media. Experimental samples (anti-miR-100) were transfected with a concentration of 50 nM anti-miR-100 miRIDIAN microRNA Hairpin Inhibitor (Thermo Fisher Scientific) and negative control samples (anti-negative) were treated with 50 nM miRIDIAN microRNA Hairpin Inhibitor Negative Control. Mock transfection samples were treated with 1X PBS. Following transfection, samples were incubated for 4 hours before adding 1 mL of antibiotic free complete growth media. Samples were incubated for 48 hours prior to harvesting.

Wound healing assay

MCF-7_M cells were seeded in 6-well plates at 3.5×10^5 cells per well in regular growth medium. Cells were allowed to adhere to the plate overnight, then transfected with antisense inhibitors to miR-100 (and a scrambled negative control) using the protocol described above. Confluent samples were scraped using sterile p-200 pipette tips 36 hours post-transfection. Samples were imaged at the indicated times post-scraping using Olympus CX41 (100X total magnification) and images were captured using Motic Images Plus 2.0. Wound healing assays were performed in duplicate, four separate times using independent cell populations.

Results

IGF1R and miR-100 are inversely expressed in breast cancer cells

Previous studies suggested that miR-100 is upregulated in triple negative and invasive human breast cancer cell lines compared to noninvasive cells (Guttilla et al., 2012; Luo et al., 2013). Semi-quantitative endpoint PCR was used to measure the levels of miR-100 in MCF-7 and MCF-7_M cells. MiR-100 was significantly upregulated in MCF-7_M cells compared to MCF-7 cells, which lacked detectable levels of miR-100 (Fig. 1a). Additional microRNAs were measured to confirm the luminal and mesenchymal phenotypes in MCF-7 and MCF-7_M cells, respectively. MiR-200c is a marker of epithelial cancer cells since miR-200c blocks EMT by targeting ZEB1/2, thereby maintaining high levels of E-cadherin (Luo et al., 2013). The high levels of miR-200c in the MCF-7 cells confirm the cells are a valid indicator of molecular changes prior to EMT. Conversely, miR-221 is a pro-EMT microRNA because it represses expression of ERα, and was therefore used as a control for MCF-7_M cells post-EMT. A control microRNA, miR-138, was expressed at equal levels in both cell lines (Fig. 1a). IGF1R has been validated as a target of miR-100 in H295R human adrenocortical carcinoma cells and A673 Ewing's sarcoma cells, and was therefore pursued as a potential target gene of miR-100 in breast cancer cells (Doghman et al., 2010; Sachdev and Yee, 2001). Both IGF1R mRNA and protein were significantly downregulated in MCF-7_M cells compared to MCF-7 cells, suggesting a negative correlation between IGF1R expression and miR-100 (Fig. 1b).

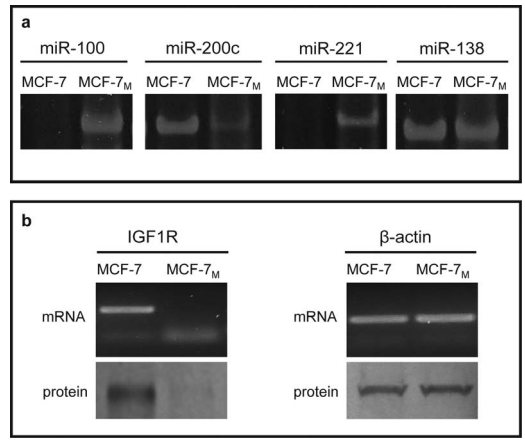

Figure 1. MiR-100 and IGF1R are inversely expressed in MCF-7 and MCF-7_M cells. a) Semi-quantitative endpoint RT-PCR was used to measure levels of the indicated microRNAs. MiR-200c and miR-221 were used as markers for luminal and mesenchymal type cells, respectively. MiR-138 was measured as a control microRNA. PCR products were resolved on a 10% non-denaturing acrylamide gel and stained with Sybr Gold. b) IGF1R and β-actin levels were measured using semi-quantitative endpoint RT-PCR (top panels) and western blot analysis (bottom panels).

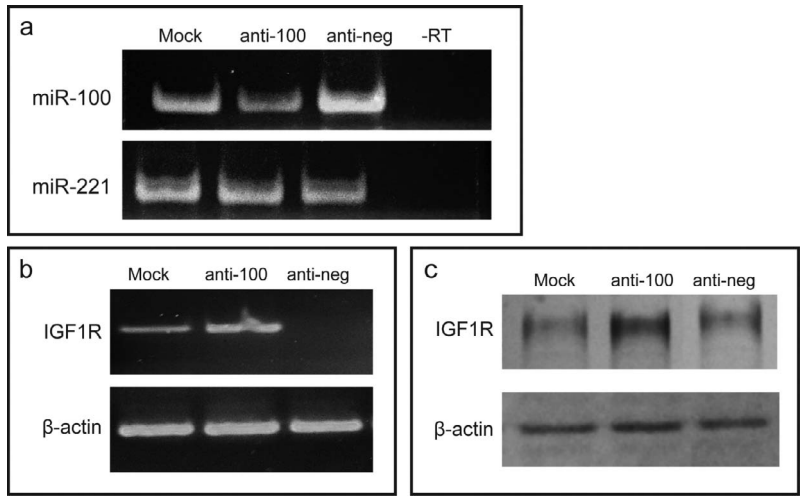

Figure 2. Endogenous IGF1R mRNA and protein levels are regulated by miR-100. Semi-quantitative endpoint RT-PCR was used to measure levels of miR-100 (a) and IGF1R mRNA (b) in MCF-7_M cells following transfection with antisense RNA targeting miR-100 (anti-100) or a negative scrambled antisense control (anti-neg). MiR-221 (a) and β-actin (b) were used as loading controls. C) IGF1R protein was measured 48 hours post-transfection using western blot analysis. β-actin was used as a loading control.

IGF1R is targeted by miR-100 in MCF-7_M cells

To confirm that IGF1R is a direct target of miR-100 in breast cancer cells, changes in IGF1R expression were measured following inhibition of miR-100 in MCF-7_M cells. Semi-quantitative endpoint PCR confirmed that miR-100 was successfully inhibited following transfection with an antisense RNA targeting miR-100 (anti-100; Fig. 2a). Anti-miR-100 treated MCF-7_M cells displayed an increase in endogenous IGF1R mRNA and protein levels compared to both mock transfection and MCF-7_M samples treated with a scrambled control antisense miRNA inhibitor (Fig. 2b, c). These data suggest that miR-100 regulates IGF1R expression by degrading IGF1R mRNA.

Loss of miR-100 inhibits migration in MCF-7_M cells

Overexpression of IGF1R increases E-cadherin mediated adhesion in MCF-7 cells (Cavallara and Christofori, 2004). Therefore, the downregulation of IGF1R by miR-100 in MCF-7_M cells is expected to weaken adhesion allowing for enhanced migration rates (Mauro et al., 2001; Pennisi et al., 2002). A wound healing assay was used to assess the role of miR-100 in regulating migration in MCF-7_M cells. MCF-7_M cells were transfected with antisense RNA targeting miR-100 (anti-100), and wounds were created 36 hours post-transfection. Anti-miR-100 treated cells exhibited a decreased migration rate compared to mock treatments and treatment with a scrambled control antisense inhibitor 24 – 36 hours following the initiation of the wound (Fig. 3). To assess the effect of miR-100 on cell proliferation, viable MCF-7_M cells were counted 24 and 48 hours post transfection with antisense RNA to miR-100. No significant differences in cell counts were present, indicating that miR-100 has no effect on proliferation rate in MCF-7_M cells (data not shown).

Discussion

EMT is orchestrated by a variety of molecular changes, including changes in microRNA expression (Theiry and Sleeman, 2006; Zhang and Ma, 2012). In this study, miR-100 was found to be strongly upregulated in MCF-7_M cells compared to MCF-7 cells, indicating a possible connection between miR-100 and EMT

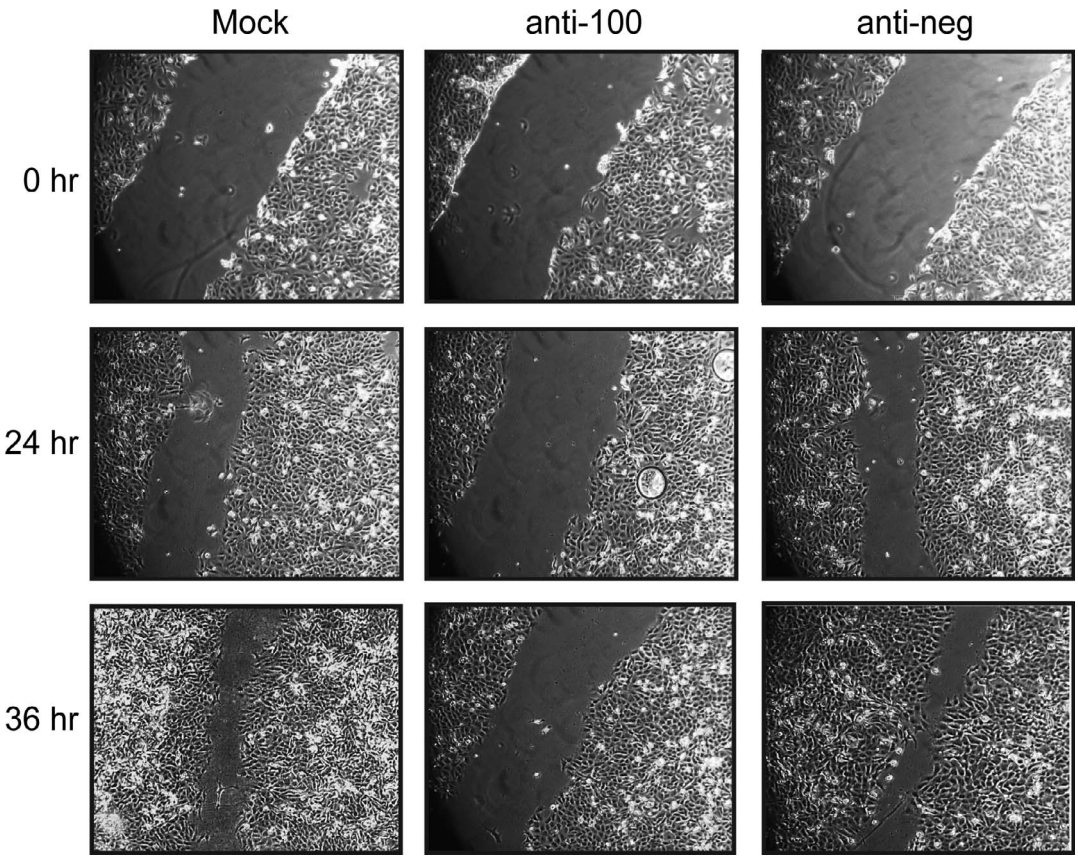

Figure 3. Inhibition of miR-100 decreases migration in MCF-7_{M} cells. A wound healing assay was performed following transfection with antisense RNA targeting miR-100 (anti-100) or a negative scrambled antisense control (anti-neg). Cells were scraped using a sterile pipet tip 36 hours post-transfection, and cells were imaged at indicated time points following initiation of the wound.

in breast cancer. It is hypothesized that miR-100 regulates migration rate in mesenchymal cells following EMT by targeting IGF1R. Strong adhesion maintains an epithelial phenotype and decreases the metastatic potential of cells. Previous studies have shown that IGF1R enhances E-cadherin mediated adhesion in MCF-7 cells (Mauro et al., 2001; Pennisi et al., 2002; Sachdev and Yee, 2001), and that IGF-1 induced cell adhesion was blocked by a mutant IGF1R receptor (Mauro et al., 2001). Therefore, miR-100 may be used to suppress endogenous IGF1R to promote a weakened adhesion network. In epithelial breast cancer cells, the insulin-like growth factor signaling pathway may be used to maintain the transformed state, but overexpression of IGF1R also maintains adhesion of the E-cadherin complex to the actin cytoskeleton. During EMT, miR-100 may repress IGF1R levels to increase invasive potential by regulating cell migration in MCF-7_{M} cells. This hypothesis is further supported by a recent study describing the potential involvement of miR-100 in EMT in breast cancer. Chen et al. reported the EMT-inducing capabilities of miR-100, despite its negative impact on tumorigenesis suggesting that some pro-tumorigenic factors are not beneficial for the metastatic phenotype (Chen et al., 2014). Our data support this observation since downregulation of IGF1R via miR-100 is one mechanism that supports the EMT-facilitating role of miR-100.

A decrease in IGF1R via miR-100 upregu-

lation indicates a reduction in the IGF signaling pathway in MCF-7_M cells. Gebeshuber et al. provided evidence that one of the ligands of IGF1R, IGF-2, is also targeted by miR-100, further suggesting an attenuation of the IGF signaling pathway in MCF-7_M cells (Gebeshuber and Martinez, 2013). Because the IGF pathway is heavily involved with cell survival and proliferation (Sachdev and Yee, 2001), the subsequent loss of IGF1R by miR-100 upregulation in EMT may explain why miR-100 has no effect on cell proliferation in MCF-7_M cells. Other recent research has focused on the correlation between obesity and risk of breast cancer. Evidence of the link between obesity and breast cancer incidence is supported by increased levels of circulating insulin and IGF-1/2, which can fuel hormone responsive cancers such as breast and endometrial cancer (Gunter et al., 2008; Lorincz and Sukumar, 2006; Renehan et al., 2006).

Overexpression of platelet derived growth factor receptors (PDGFRs) in response to a decrease in IGF1R may allow MCF-7_M cells to maintain cell survival even in the presence of reduced IGF signaling (Campbell and Moorehead, 2011; Jechlinger et al., 2006). An inverse correlation between PDGFR and IGF1R expression suggests that regulation of IGF1R by miR-100 may have an additional off target effect of upregulating PDGFR. Breast cancer cells can upregulate PDGFR in direct response to loss of IGF1R (Lorincz and Sukumar, 2006). Not only is PDGFR upregulated in EMT, but this increase in PDGF signaling is also correlated with increased invasiveness, migration, and drug resistance in breast cancer (Campbell and Moorehead, 2011; Jechlinger et al., 2006; Thomson et al., 2011). The combination of decreased adhesion and overexpression of PDGFR may promote EMT and increase the risk of metastasis. A potential interaction between PDGFR and miR-100 will be investigated in future studies.

Although these data support the hypothesis that miR-100 regulates migration in MCF-7_M cells, it is unclear if the decrease in IGF1R is sufficient to induce this phenotype. While IGF1R is a strong candidate, there are potentially additional target genes of miR-100 that may promote and/or maintain EMT. Furthermore, the mechanism driving the upregulation of miR-100 during EMT it is still unclear. EMT is believed to be the first step in metastasis, therefore inhibiting miR-100 may be a potential therapy for preventing or reversing EMT. Profiling molecular changes in EMT may be used as biomarkers for monitoring patients and designing treatment plans. Since most therapies effective against Luminal A tumors are unsuccessful in fighting Basal-like tumors, understanding the molecular makeup of these tumors may lead to more efficient treatment options. Furthermore, understanding miR-100 and IGF1R expression during cancer progression may elucidate factors contributing to drug-resistance or reoccurrence in tumors.

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References

Campbell, C.I. and Moorehead, R.A. (2011). Mammary tumors that become independent of the type I insulin-like growth factor receptor express elevated levels of platelet-derived growth factor receptors. BMC Cancer 11, 480.

Cavallara, U. and Christofori, G. (2004), Cell adhesion and signaling by cadherins and Ig-CAMs in cancer. Nat. Rev. Cancer 4, 118–132.

Chen, D., Sun, Y., Yuan, Y., Han, Z., Zhang, P., Zhang, J., You, M.J., Teruya-Feldstein, J., Wang, M., Gupta, S., et al. (2014). miR-100 induces epithelial-mesenchymal transition but suppresses tumorigenesis, migration, and invasion. PLoS Genet. 10, e1004177.

Doghman, M., El Wakil, A., Cardinaud, B., Thomas, E., Wang, J., Zhao, W., Peralta-Del Valle, M.H., Figueiredo, B.C., Zambetti, G.P., and Lalli, E. (2010). Regulation of insulin-like growth factor-mammalian target of rapamycin signaling by microRNA in childhood adrenocortical tumors. Cancer Res. 70, 4666–4675.

Farazi, T.A., Hoell, J.I., Morozov, P., and Tuschl, T. (2013). MicroRNAs in human cancer. Adv. Exp. Med. Biol. 774, 1–20.

Gebeshuber, C.A. and Martinez, J. (2013). miR-100

suppresses IGF2 and inhibits breast tumorigenesis by interfering with proliferation and survival signaling. Oncogene 32, 3306–3310.

Gunter, M.J., Hoover, D.R., Yu, H., Wassertheil-Smoller, S., Manson, J.E., Li, J., Harris, T.G., Rohan, T.E., Xue, X., Ho, G.Y., et al. (2008). A prospective evaluation of insulin and insulin-like growth factor-I as risk factors for endometrial cancer. Cancer Epidemiol. Biomarkers Prev. 17, 921–929.

Guttilla, I.K., Pheonix, K.N., Hong, X., Tirnauer, J.S., Claffey, K.P., and White, B.A. (2012). Prolonged mammosphere culture of MCF-7 cells induces EMT and repression of the estrogen receptor by microRNAs. Breast Cancer Res. Treat. 132, 75–85.

Iorio, M.V. and Croce, C.M. (2009). MicroRNAs in cancer: small molecules with a huge impact. J. Clin. Oncol. 27, 5848–5856.

Jechlinger, M., Sommer, A., Moriggl, R., Seither, P., Kraut, N., Capodiecci, P., Donovan, M., Cordon-Cardo, C., Beug, H., and Grünert, S. (2006). Autocrine PDGFR signaling promotes mammary cancer metastasis. J. Clin. Invest. 116, 1561–1570.

Kittaneh, M., Montero, A.J., and Glück, S. (2013). Molecular profiling for breast cancer: a comprehensive review. Biomark. Cancer 5, 61–70.

Lorincz, A.M. and Sukumar, S. (2006). Molecular links between obesity and breast cancer. Endocr. Relat. Cancer 13, 279–292.

Luo, D., Wilson, J.M., Harvel, N., Liu, J., Pei, L., Huang, S., Hawthorn, L., and Shi, H. (2013). A systematic evaluation of miRNA:mRNA interactions involved in the migration and invasion of breast cancer cells. J. Transl. Med. 11, 57.

Mauro, L., Bartucci, M., Morelli, C., Andò, S., and Surmacz, E. (2001). IGF-1-receptor-induced cell-cell adhesion of MCF-7 breast cancer cells requires the expression of junction protein ZO-1. J. Biol. Chem. 276, 39892–39897.

McKinsey, E.L., Parrish, J.K., Irwin, A.E., Niemeyer, B.F., Kern, H.B., Birks, D.K., and Jedlicka, P. (2011). A novel oncogenic mechanism in Ewing sarcoma involving IGF pathway targeting by EWS/Fli1-regulated microRNAs. Oncogene 30, 4910–4920.

Moody, S.E., Perez, D., Pan, T.C., Sarkisian, C.J., Portocarrero, C.P., Sterner, C.J., Notorfrancesco, K.L., Cardiff, R.D., and Chodosh, L.A. (2005). The transcriptional repressor Snail promotes mammary tumor recurrence. Cancer Cell 8, 197–209.

Pennisi, P.A., Barr, V., Nunez, N.P., Stannard, B., and Le Roith, D. (2002). Reduced expression of insulin-like growth factor I receptors in MCF-7 breast cancer cells leads to a more metastatic phenotype. Cancer Res. 62, 6529–6537.

Peppercorn, J., Perou, C.M., and Carey, L.A. (2008). Molecular subtypes in breast cancer evaluation and management: divide and conquer. Cancer Invest. 26, 1–10.

Renehan, A.G., Frystyk, J., and Flyvbjerg, A. (2006). Obesity and cancer risk: the role of the insulin-IGF axis. Trends Endocrinol. Metab. 17, 328–336.

Sachdev, D. and Yee, D. (2001). The IGF system and breast cancer. Endocr. Relat. Cancer 8, 197–209.

Sporn, M.B. (1996). The war on cancer. Lancet. 347, 1377–1381.

Theiry, J.P. and Sleeman, J.P. (2006). Complex networks orchestrate epithelial-mesenchymal transitions. Nat. Rev. Mol. Cell Biol. 7, 131–142.

Thomson, S., Petti, F., Sujka-Kwok, I., Mercado, P., Bean, J., Monaghan, M., Seymour, S.L., Argast, G.M., Epstein, D.M., and Haley, J.D. (2011). A systems view of epithelial-mesenchymal transition signaling states. Clin. Exp. Metastasis 28, 137–155.

Wang, Y. and Zhou, B.P. (2013). Epithelial-mesenchymal transition—a hallmark of breast cancer metastasis. Cancer Hallm. 1, 38–49.

Zhang, J. and Ma, L. (2012). MicroRNA control of epithelial-mesenchymal transition and metastasis. Cancer Metastasis Rev. 31, 653–662.

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