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Effects of SDF-1-CXCR4 signaling on microRNA expression and tumorigenesis in estrogen receptor-alpha (ER- α)-positive breast cancer cells

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Abstract

The majority of breast cancer cases ultimately become unresponsive to endocrine therapies, and this progression of breast cancer from hormone-responsive to hormone-independent represents an area in need of further research. Additionally, hormone-independent carcinomas are characterized as being more aggressive and metastatic, key features of more advanced disease. Having previously shown the ability of the stromal-cell derived factor-1 (SDF-1)-CXCR4 signaling axis to promote primary tumorigenesis and hormone independence by overexpressing CXCR4 in MCF-7 cells, in this study we further examined the role of SDF-1/CXCR4 in the endogenously CXCR4-positive, estrogen receptor α (ER- α)-positive breast carcinoma cell line, MDA-MB-361. In addition to regulating estrogen-induced and hormone-independent tumor growth, CXCR4 signaling stimulated the epithelial-to-mesenchymal transition, evidenced by decreased CDH1

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Conflict of interest

The authors declare no conflicts of interest.

expression following SDF-1 treatment. Furthermore, inhibition of CXCR4 with the small molecule inhibitor AMD3100 induced CDH1 gene expression and inhibited CDH2 gene expression in MDA-MB-361 cells. Further, exogenous SDF-1 treatment induced ER- α -phosphorylation in both MDA-MB-361 and MCF-7-CXCR4 cells, demonstrating ligand-independent activation of ER- α through CXCR4 crosstalk. qPCR microRNA array analyses of the MDA-MB-361 and MCF-7-CXCR4 cell lines revealed changes in microRNA expression profiles induced by SDF-1, consistent with a more advanced disease phenotype and further supporting our hypothesis that the SDF-1/CXCR4 signaling axis drives ER- α -positive breast cancer cells to a hormone independent and more aggressive phenotype. In this first demonstration of SDF-1-CXCR4-induced microRNAs in breast cancer, we suggest that this signaling axis may promote tumorigenesis via microRNA regulation. These findings represent future potential therapeutic targets for the treatment of hormone-independent and endocrine-resistant breast cancer.

Keywords

SDF-1; CXCR4; microRNA; breast carcinoma; hormone independence; AMD3100

Introduction

Chemokines are a family of structurally related glycoproteins, originally described as molecules mediating chemotactic events [1, 2]. Stromal cell-derived factor-1 (SDF-1), also known as CXCL12, is a member of the CXC chemokine subfamily and the only known ligand for CXC chemokine receptor 4 (CXCR4). Though involved in many biological processes, the SDF-1-CXCR4 signaling axis has been shown to play important roles in breast cancer [2, 3]. CXCR4 is overexpressed in both primary invasive and *in situ* ductal carcinomas, suggesting an important role for the SDF-1-CXCR4 axis at all stages of the disease [4]; however, the impact of CXCR4 signaling in primary breast tumorigenesis remains to be clearly defined.

Estrogen receptor alpha (ER- α) status is a widely used prognostic marker of breast carcinoma, and it has long been known that estrogen has the ability to promote breast tumor formation and proliferation [5, 6]. Inhibition of ER- α signaling abrogates the tumor promoting effects of estrogen [5, 7–10]; these effects are responsible for the successful application of targeted therapies such as tamoxifen, fulvestrant (ICI 182,780), and aromatase inhibitors. Despite the effectiveness of these therapies, approximately half of ER- α -positive breast cancer patients exhibit *de novo* resistance, while those initially responsive will eventually develop resistance [11]. The progression to endocrine-resistance and hormone-independence represent hallmarks of progressive carcinoma [12, 13]. We have recently demonstrated the ability of CXCR4 overexpression to promote hormone-independent tumorigenesis in the normally ER- α (+), estrogen-dependent MCF-7 breast carcinoma cell line [14]. SDF-1 is a known ER- α -mediated gene, and our data as well as others, supports the existence of an ER- α —SDF-1/CXCR4 crosstalk [14, 15], which may strongly contribute to the progression to hormone independence.

In addition to being overexpressed in a number of malignant cancers including breast, CXCR4 is a known mediator of metastasis [3, 16–18]. The pro-metastatic effects of SDF-1/CXCR4 signaling in breast cancer can be inhibited through the use of blocking antibodies, small molecule inhibitors, as well as heparin oligosaccharides [4, 14, 19]. Further, SDF-1 and CXCR4 expression have been associated with the epithelial-to-mesenchymal transition (EMT) phenotype, characterized by the loss of epithelial markers (E-cadherin, Zo-1) and the gain of mesenchymal surface markers (N-cadherin, vimentin), a key step in the progression to a metastatic phenotype [14, 20, 21]. In addition, EMT has been shown to be regulated by

microRNAs (miRNA) [22–24], small non-coding RNA (18–22 nucleotides) that downregulate the expression of target genes by degradation of mRNA or inhibition of translation [25]. Despite evidence of other chemokines mediating miRNA expression as well as miRNA targeting of chemokine signaling [26–28], the effects of SDF-1-CXCR4 signaling on miRNA expression in breast cancer have not yet been examined. This is of particular interest in the area of breast cancer research as the SDF-1-CXCR4 axis is emerging not only as a regulator of cell metastasis, but also in primary cancer tumorigenesis, hormone independence, and disease progression [4, 14]. Insight into the mechanism of SDF-1-CXCR4 action in breast cancer may provide future therapeutic targets for the development of novel cancer treatments.

The purpose of this study was to investigate the effects of CXCR4 signaling on primary tumorigenesis, EMT phenotype, and regulation of ER- α phosphorylation in the endogenously ER- α (+)/CXCR4 (+) breast carcinoma cell line MDA-MB-361. To gain further mechanistic insight into the SDF-1-CXCR4 axis, we compared miRNA profiles of MDA-MB-361 cells with an MCF-7 cell line artificially overexpressing CXCR4 [14]. This is the first report of hormone-dependent and -independent regulation of MDA-MB-361 tumorigenesis by the SDF-1-CXCR4 axis and provides compelling evidence that SDF-1 induces gene, protein, and miRNA expression changes consistent with a more aggressive phenotype.

Materials and Methods

Cells and Reagents

The MDA-MB-361 cell line (ER- α -positive human breast cancer cell line) was acquired from ATCC. The MDA-MB-361 cell line was chosen for these studies due to their ER- α -positive status as well as high basal level expression of CXCR4 [14]. The MCF-7 cell line overexpressing CXCR4 was generated as previously published [14], and cells were cultured as previously described [29, 30]. Anti-CXCR4 blocking antibody was purchased from R&D Systems (Minneapolis, MN) and AMD3100 from Sigma-Aldrich (St. Louis, MO).

Animal studies

Primary xenograft tumor studies were performed as described [29, 30]. MDA-MB-361 cells were harvested and viable cells mixed with Matrigel Reduced Factors (BD Biosciences, San Jose, CA). 5×10^6 cells were injected bilaterally into the mammary fat pad of 4–6 week old ovariectomized female Nu/Nu mice. Tumor size was monitored by digital caliper and tumor volume calculated with the formula: $4/3\pi LM^2$ (L=larger radius, M=smaller radius). Anti-CXCR4 treatment groups were injected with cells mixed with 50ul matrigel containing anti-CXCR4 blocking antibody (75 ng/injection) or IgG control. AMD3100 treatment experiment animals were injected intraperitoneally (i.p.) with AMD3100 (5 mg/kg/animal) suspended in DMSO and PBS (1:5) once daily for the duration of the study. Specific treatment start dates are indicated in the corresponding figure legend. Experimental groups, n=5. All procedures involving animals were conducted in compliance with State and Federal laws, U.S. Department of Health and Human Services, and guidelines established by Tulane University Animal Care and Use Committee. The facilities and laboratory animal programs of the University are accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

RNA Isolation and Quantitative Realtime PCR

Total RNA was isolated from cultured cells using RNeasy (Qiagen, Valencia, CA) following manufacturer's protocol and the quantity and quality determined by absorbance (260, 280nm). 2ug total RNA was reverse-transcribed (iScript kit; BioRad Laboratories, Hercules,

CA) and analyzed by real-time PCR [31]. Primer sequences are as follows (Invitrogen, Carlsbad, CA): β -Actin (5'-TGAGCGCGGCTACAGCTT-3'; 5'-CCTTAATGTACACACGATT-3'), CXCR4 (5'-GCATGACGGACAAGTACAGGCT-3'; 5'-AAAGTACCAGTTTGCCACGGC-3'), CDH1 (5'-AGGTGACAGAGCCTCTGGATAGA-3'; 5'-TGGATGACACAGCGTGAGAGA-3'), CDH2 (5'-GCCCCTCAAGTGTACCTCAA-3'; 5'-AGCCGAGTGATGGTCCAATTT-3'), forward and reverse, respectively.

Reverse Transcriptase-Polymerase Chain Reaction

2ug total RNA (above) was used to synthesize cDNA transcribed with SuperScript III (Invitrogen, Carlsbad, CA) and mRNA amplified. Primer sequences are as follows (Invitrogen, Carlsbad, CA): CXCR4 (5'-AGTATATACACTTCAGATAAC-3'; 5'-CCACCTTTTCAGCCAACAG-3'); SDF-1 (5'-GCCAGAGCCAACGTCAAGCATCTC-3'; 5'-GGCAAAGTGTCCAAAACAAAGCCC-3'); PgR (5'-GAATTTAGCGGGGATCCA-3'; 5'-TGCCACACTTCGATTGT-3'); BCL2 (5'-CGCCCTGTGGATGACTGAGT-3'; 5'-GGGCCGTACAGTTCCACAA-3'); VEGF (5'-GCAGAAGGAGGAGGGCAGAATC-3'; 5'-GGCACACAGGATGGCTTGAAGATG-3'); GAPDH (5'-ACAGTCAGCCCGCATCTTCTT-3'; 5'-GACAAGCTTCCCGTTCTCAG-3').

CDH1 ELISA

The human sE-Cadherin ELISA was carried out according to the manufacturers' protocol. Briefly, MDA-MB-361 cells (10^4 cells/well) were plated overnight and treated for 72h with SDF-1 (100 ng/ml) or vehicle control. After cell lysis and centrifugation, the cytoplasmic fractions were diluted 1:1 with calibrator diluent and the level of E-cadherin (CDH1) determined by sE-Cadherin ELISA (DCADE0; R&D Systems, Minneapolis, MN). The absorbance was read on a Bio-Tek Synergy plate reader (Winooski, VT) at 450 nm. Data represented as mean percent control $\pm$ SEM. Assays were run in triplicate with internal duplicate drug treatments.

Western Blot Analysis

Western blot analyses were conducted as published [32]. After 72hrs in phenol red-free DMEM medium supplemented with 5% charcoal-stripped fetal bovine serum (CS-FBS), cells were refed with medium containing SDF-1 (100 ng/ml). Cells were harvested at the indicated time points (0 – 120 minutes) in PBS/EDTA. Membranes were probed with primary antibodies according to manufacturer's protocol. Antibodies: phospho S118, phospho S167 (Cell Signaling Technology, Danvers, MA), total ER- α , Rho-GDI (Santa Cruz Biologicals, Santa Cruz, CA). IR-tagged secondary antibodies were purchased from Li-Cor Biosciences. Blots were analyzed by the Odyssey Infrared Imaging System (Li-Cor Biosciences, Lincoln, NE). Experiments were conducted in triplicate.

microRNA qPCR array

The Human Cancer microRNA RT² Profiler™ PCR Arrays (MAH-102A) were obtained from SABiosciences (Frederick, MD). MDA-MB-361 or MCF-7-CXCR4 cells were plated in 5% CS DMEM at 50% confluency and treated 24 hours later with SDF-1 (100 ng/ml) or vehicle for 24 hours. Cells were harvested and total RNA, including small RNA, was isolated using the miRNeasy kit per manufacturer's instructions (Qiagen, Valencia, CA). The quantity and quality of the RNA were determined by absorbance at 260 and 280 nm using the NanoDrop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE). Total RNA (1.5 μ g) was reverse-transcribed using the miRNA RT² First Strand cDNA synthesis kit following manufacturer's protocol (SABiosciences, Frederick, MD) and assayed via an

optimized, real-time RT-PCR reaction for expression of 84 miRNA genes related to cancer regulation according to the manufacturer's protocol. Experiments were run in triplicate.

Statistical Analysis

Studies involving >2 groups analyzed by one-way ANOVA with Tukey's post-test; all others were subjected to unpaired student's t-test (Graph Pad Prism V.4). p-values < 0.05 were considered statistically significant.

Results

CXCR4 signaling regulates estrogen-stimulated MDA-MB-361 tumorigenesis

CXCR4 gene expression levels were confirmed by qPCR in the MDA-MB-361 breast cancer cell line (3.62 ± 0.82 fold, $p < 0.05$) compared to the known ER- α (+)/CXCR4^{low} MCF-7 cell line (set to 1) and the ER- α (-)/CXCR4^{high} MDA-MB-231 cell line (5.09 ± 1.14 fold, $p < 0.05$) (Figure 1A). RT-PCR analysis of cells treated with estrogen (100pM) for 18 hours revealed increased levels of ER- α -mediated genes including PgR, BCL2, and VEGF-A (Supplemental Figure 1A) confirming intact ER- α signaling in our MDA-MB-361 cell system. Additionally, SDF-1, an ER- α -inducible gene [33], was increased 4.8 ± 0.34 fold ($p < 0.001$) in response to estrogen stimulation (Supplemental Figure 1B), further demonstrating a link between ER- α signaling and the SDF-1/CXCR4 axis.

We have previously shown that artificial CXCR4 overexpression enhances tumor growth in the ER- α -dependent MCF-7 breast carcinoma cell line [14]. Therefore, having confirmed endogenous expression of CXCR4 in the MDA-MB-361 cell line, the effects of CXCR4 signaling on MDA-MB-361 tumorigenesis were examined using our well established xenograft tumor model [14]. 4–6 week old ovariectomized female nude mice supplemented with 17 β -estradiol pellets (0.72 mg, 60 day release) were injected with MDA-MB-361 cells in the mammary fat pad (MFP). Results revealed decreased estrogen-stimulated tumor volume in animals injected with MDA-MB-361 cells mixed with matrigel containing anti-CXCR4 blocking antibody (57.7 ± 23.7 mm³, $p < 0.01$) compared to isotype control (164.8 ± 19.8 mm³; Figure 1B) on day 46 post cell injection, as well as animals treated with daily injections of AMD3100 (159.4 ± 19.0 mm³, $p < 0.05$) compared to vehicle control animals (241.2 ± 37.8 mm³; Figure 1C) on day 25 post cell injection. These results reveal a role for SDF-1/CXCR4 signaling in hormone-driven tumorigenesis of MDA-MB-361 cells.

CXCR4 expression drives hormone-independent MDA-MB-361 tumorigenesis

Though MDA-MB-361 cells remain estrogen-responsive both *in vitro* and *in vivo*, these cells also possess the ability to grow independently of estrogen in a xenograft model (Supplemental Figure 1C). Previous work from our lab demonstrated the ability of CXCR4 overexpression to induce hormone-independent tumorigenesis in an artificial system [14]; therefore, the effect of CXCR4 signaling on MDA-MB-361 hormone-independent tumorigenesis was also examined. 4–6 week old ovariectomized female nude mice were injected with MDA-MB-361 cells in the MFP in the absence of estrogen. Following detectable tumor formation (day 26 post cell injection), animals were randomized into treatment groups which received daily i.p. injections of the CXCR4-specific inhibitor AMD3011 (5mg/kg) or vehicle control for 15 days. As shown in figure 2A, at day 41 post cell injection, tumor volume in animals treated with AMD3100 was significantly decreased (33.6 ± 7.8 mm³, $p < 0.05$) compared to vehicle treated animals (73.6 ± 11.1 mm³).

CXCR4 signaling promotes the epithelial-to-mesenchymal transition

The SDF-1/CXCR4 axis is a known mediator of cell migration and metastasis [1, 3, 34, 35] and the expression of CXCR4 is correlated with decreased patient survival [14, 36, 37].

Furthermore, the acquisition of mesenchymal characteristics through the process known as epithelial-to-mesenchymal transition (EMT) has been associated with increased migration, invasion, and metastasis [38]. Therefore, we next examined the role of CXCR4 signaling on EMT-related genes by qPCR analyses. Inhibition of CXCR4 by AMD3100 (5 ug/ml) in MDA-MB-361 cells for 48 hours resulted in increased expression of CDH1 (2.32 ± 0.05 fold, $p < 0.05$), a well known epithelial cell marker, and decreased expression of CDH2 (-1.44 ± 0.39 fold, $p < 0.01$), a mesenchymal cell marker, compared to vehicle treated cells (normalized to 1; Figure 2B). Additionally, as illustrated in figure 2C, SDF-1 treatment (100 ng/ml) for 72 hours was sufficient to decrease CDH1 protein expression by 25% compared to controls as determined by ELISA (from 100% to $74.94 \pm 0.9398\%$; $p < 0.001$).

SDF-1 induces ER phosphorylation and microRNA expression changes concordant with a more advanced tumor phenotype

To determine if the effects of CXCR4 signaling on hormone-independence were mediated through activation of the ER, western blot analysis for phosphorylated ER- α was conducted. Analysis of MDA-MB-361 cells (Figure 3A) and our previously characterized MCF-7 cell line stably overexpressing CXCR4 (MCF-7-CXCR4, Figure 3B) revealed a time-dependent increase in ER- α phosphorylation at both S118 and S167 following SDF-1 treatment (100 ng/ml). These data suggest the hormone-independent phenotype observed may be due, at least in part, to the existence of an SDF-1/CXCR4-ER- α crosstalk, in both an artificial (MCF-7-CXCR4) and an endogenous (MDA-MB-361) cell system, consistent with previous reports [14, 15].

Altered miRNA expression is a common characteristic of many cancers, including breast, and their effects, both oncogenic and tumor suppressive, are exerted via regulation of many processes including tumorigenesis [39], hormone independence and endocrine resistance [40–43], EMT [44] and metastasis [45]. miRNA qPCR superarray analyses of MDA-MB-361 cells (Figure 3C) and MCF-7-CXCR4 cells (Figure 3D) treated with SDF-1 for 24hrs revealed a number of changes in the miRNA expression compared to vehicle treated cells. Clustergrams shown in figure 3B–C illustrate miRNA expression changes for 3 independent samples. Table 1 displays the miRNAs found to have significantly ($p < 0.01$) changed expression in response to SDF-1 treatment (miRNAs commonly altered between both cell lines are listed first).

Discussion

CXCR4 expression is highly correlated with decreased breast carcinoma patient survival [14, 36, 37] and the SDF-1-CXCR4 axis is a known regulator of cancer metastasis [34–37, 46, 47]. Consistent with the role of CXCR4 in cancer cell proliferation, survival and metastasis, patient data now show that persons diagnosed with CXCR4(+) tumors have a significantly worse survival prognosis than those with CXCR4(–) tumors, independent of ER status [14, 36]. Studies examining CXCR4 expression in clinical breast carcinoma samples revealed CXCR4 expression in both primary invasive breast carcinomas as well as ductal carcinoma *in situ* (DCIS), suggesting a role for the SDF-1-CXCR4 axis at all stages of the disease [4]. Despite numerous studies on the SDF-1-CXCR4 axis, its role in primary tumorigenesis is not fully understood. Using the endogenously CXCR4 and ER- α -positive MDA-MB-361 breast carcinoma cell line in addition to our MCF-7-CXCR4 cell line, we have shown here that inhibition of SDF-1-CXCR4 signaling reduces both hormone-independent and estrogen-induced tumorigenesis *in vivo*. Furthermore, we show the ability of SDF-1 treatment to induce phosphorylation of the ER- α at both S118 and S167, indicating ligand-independent activation, and further supporting the existence of SDF-1/CXCR4-ER- α crosstalk in the regulation of hormone-independence.

Analysis of miRNAs regulated by SDF-1 using qPCR revealed key miRNAs involved in ER- α regulation. miRNAs miR-222, miR-206, and miR-18b, miRNAs previously shown by us and others to target ER- α [48–50], were aberrantly expressed following stimulation with SDF-1. Importantly, miR-222 is not only a known repressor of ER- α gene expression, but has also been implicated in establishing resistance to both tamoxifen [51] and, more recently, fulvestrant [41]. It has been suggested that miR-222 is involved in the transition from an ER- α (+) status to an ER- α (–) one, indicative of the progression to a more advanced phenotype [49]. Furthermore, SDF-1 induced the expression of miRNAs commonly shown to be upregulated in ER- α (–) breast cancer profiles, including miR-222, miR-206, and miR-181d [49, 52]. Taken together, these data suggest that the SDF-1-CXCR4 axis mediates hormone independence and the progression to a more aggressive phenotype through mechanisms including miRNA expression changes and ER- α regulation.

While progression to hormone independence is a hallmark of advanced breast carcinomas which ultimately progress to more invasive and metastatic phenotypes [53], and the link between SDF-1/CXCR4 expression and breast cancer metastasis has been clearly documented [3, 16, 35], we demonstrate here that SDF-1 also stimulates the EMT, a key feature of metastatic cells, through decreased expression of the epithelial marker CDH1. Furthermore, inhibition of CXCR4 signaling with AMD3100 revealed inverse effects with increased CDH1 gene expression and decreased expression of CDH2, a mesenchymal cell marker. While the precise mechanism is currently unknown, we believe these data, along with our previously published work [14], suggest SDF-1/CXCR4 regulation of EMT.

Regulation of miRNA expression might represent one possible mechanism underlying SDF-1 regulation of EMT. qPCR miRNA analyses revealed significant SDF-1-induced changes in expression of miRs associated with the regulation of cellular invasion and metastasis. Well established metastatic-inducing miRNAs miR-214, miR-222, and miR-373 were increased following treatment with SDF-1 [54–57]. SDF-1 also upregulated miRNAs miR-143 and miR-142-5p, which have been reported to be increased in profiling studies of metastatic versus non-metastatic tumor samples [58–60]. These data suggest that altered miRNA expression may play a role in the ability of SDF-1 to induce a more mesenchymal phenotype in MDA-MB-361 and MCF-7-CXCR4 cells.

The current study demonstrates a role for SDF-1-CXCR4 signaling in both estrogen-induced and hormone-independent tumorigenesis of the endogenously CXCR4-positive, ER- α (+) breast cancer cell line MDA-MB-361. Furthermore, SDF-1 treatment induced changes in miRNA expression, demonstrated here for the first time, consistent with hormone independence and metastasis. In addition to furthering our knowledge of the role of the SDF-1-CXCR4 axis in the progression of breast carcinoma, these data provide insight into the effects of SDF-1-CXCR4 signaling on miRNA expression, a novel alternative mechanism which may provide future therapeutic targets for the treatment of ER- α (+), CXCR4 (+), hormone-independent breast disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. CXCR4 expression drives estrogen-stimulated MDA-MB-361 tumorigenesis

(A) MDA-MB-361 cells were harvested for RNA isolation and basal expression of CXCR4 examined by qPCR compared to MCF-7 and MDA-MB-231 cells. Bars represent mean fold expression $\pm$ SEM of triplicate experiments. (B–C) Female, 4–6 week old, ovariectomized Nu/Nu mice ($n=5$ /group) were injected (MFP) with 5×10^6 MDA-MB-361 cells in 50 μ l of Matrigel. Animals were implanted with 17β -estradiol (0.72 mg, 60 day time release) pellets subcutaneously in the dorsal neck. (B) Matrigel injections contained isotype IgG or anti-CXCR4 (75 ng/injection) antibodies. (C) Following tumor formation (11 days post cell injection), animals were treated with twice daily i.p. injections of vehicle or AMD3100 (5 mg/kg/animal). Tumors were measured by digital caliper. Data represented as average tumor volume (mm³) $\pm$ SEM. *, $p < 0.05$, **, $p < 0.01$.

Figure 2. CXCR4 signaling drives MDA-MB-361 hormone-independent tumorigenesis and stimulates EMT

(A) 4–6 week old Nu/Nu ovariectomized female mice were injected (MFP) with 5×10^6 MDA-MB-361 cells mixed with matrigel (reduced factor). After tumor formation (day 26 post cell injection), animals were randomized into treatment groups and received once daily i.p. treatment with the CXCR4 inhibitor AMD3100 (5mg/kg/animal) or vehicle control. Tumors were measured by digital caliper. Data represented as average tumor volume (mm³) $\pm$ SEM. (B) qPCR analysis of EMT-related genes, CDH1 and CDH2, in MDA-MB-361 cells treated with AMD3100 (5 μ g/ml) for 48 hours. Bars represent mean fold change $\pm$ SEM of triplicate samples, vehicle normalized to 1. (C) CDH1 protein levels as detected by CDH1 ELISA of MDA-MB-361 cells treated with SDF-1 (100 ng/ml) for 72 hours. Bars represent mean percent positive $\pm$ SEM of triplicate samples, vehicle normalized to 100%. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 3. SDF-1 activates ER- α via phosphorylation and alters miR expression consistent with more advanced phenotype

(A–B) Western blot analysis for ER- α phosphorylation at serine 118 and serine 167 in a time course treatment of (A) MDA-MB-361 or (B) MCF-7-CXCR4 cells with SDF-1 (100ng/ml) following 72 hours of serum starvation. Rho GDI or α -tubulin were used as loading controls. Each gel is representative of three independent blots. (C–D) Representative clustergrams of miRNA expression changes as determined by qPCR arrays in (C) MDA-MB-361 or (D) MCF-7-CXCR4 cells following 24 hours of treatment with SDF-1 (100 ng/ml). Red indicates increased expression, while green denotes decreased expression.

Table 1

SDF-1-induced microRNA expression changes in CXCR4-positive breast carcinoma cells.

<u>MDA-MB-361</u>		
microRNA	Fold Change	p-value
18b	1.82	<0.01
96	1.39	<0.01
98	2.49	<0.05
181d	1.26	<0.05
let-7a	1.59	<0.01
let-7c	1.56	<0.01
let-7d	1.56	<0.05
let-7f	2.59	<0.05
let-7g	1.25	<0.05
124	3.14	<0.05
127-5p	1.87	<0.01
133b	4.57	<0.05
142-5p	2.12	<0.05
143	1.76	<0.05
144	5.71	<0.001
150	5.41	<0.05
206	5.91	<0.05
214	3.74	<0.05
222	1.99	<0.01
372	4.40	<0.001
373	12.61	<0.05
<u>MCF-7-CXCR4</u>		
microRNA	Fold Change	p-value
18b	1.89	<0.05
96	19.34	<0.05
98	20.53	<0.05
181d	4.54	<0.01
let-7a	24.70	<0.05
let-7c	5.23	<0.01
let-7d	2.97	<0.05
let-7f	5.71	<0.05
let-7g	15.38	<0.001
7	13.86	<0.05
15a	10.46	<0.01
15b	9.62	<0.01
16	15.67	<0.05
17	31.05	<0.01

MCF-7-CXCR4

microRNA	Fold Change	p-value
18a	4.53	<0.01
19a	26.97	<0.01
20a	34.70	<0.01
20b	17.07	<0.01
23b	11.00	<0.05
25	8.48	<0.05
27a	28.44	<0.01
27b	16.95	<0.05
29a	44.32	<0.01
30c	15.78	<0.01
34a	2.05	<0.05
92a	3.84	<0.05
100	18.51	<0.05
126	19.84	<0.05
128	7.36	<0.05
130a	2.30	<0.05
132	7.26	<0.05
138	8.96	<0.01
140-5p	14.55	<0.05
146a	2.16	<0.05
146b-5p	7.76	<0.05
148a	23.16	<0.01
148ab	21.41	<0.01
149	5.07	<0.05
181a	3.78	<0.001
181b	6.73	<0.01
181c	3.66	<0.001
183	9.11	<0.05
191	10.13	<0.05
193a-5p	3.27	<0.01
193b	7.53	<0.01
199a-3p	3.78	<0.05
200c	9.96	<0.05
205	5.04	<0.01
218	7.13	<0.05
301a	18.29	<0.01
335	5.66	<0.05
378	9.92	<0.01
let7b	3.48	<0.001
let-7e	16.22	<0.01
let-7i	7.67	<0.01