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RESEARCH ARTICLE

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Extracellular acidosis stimulates breast cancer cell motility through aryl hydrocarbon receptor and c-src kinase activation

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Abstract

A reduction in extracellular pH (pHe) is a characteristic of most malignant tumors. The aryl hydrocarbon receptor (AhR) is a transcription factor localized in a cytosolic complex with c-Src, which allows it to trigger nongenomic effects through c-Src. Considering that the slightly acidic tumor microenvironment promotes breast cancer progression in a similar way to the AhR/c-Src axis, our aim was to evaluate whether this pathway could be activated by low pHe. We examined the effect of pHe 6.5 on AhR/c-Src axis using two breast cancer cell lines (MDA-MB-231 and LM3) and mammary epithelial cells (NMuMG) and found that acidosis increased c-Src phosphorylation only in tumor cells. Moreover, the presence of AhR inhibitors prevented c-Src activation. Low pHe reduced intracellular pH (pHi), while amiloride treatment, which is known to reduce pHi, induced c-Src phosphorylation through AhR. Analyses were conducted on cell migration and metalloproteases (MMP)-2 and -9 activities, with results showing an acidosis-induced increase in MDA-MB-231 and LM3 cell migration and MMP-9 activity, but no changes in NMuMG cells. Moreover, all these effects were blocked by AhR and c-Src inhibitors. In conclusion, acidosis stimulates the AhR/c-Src axis only in breast cancer cells, increasing cell migration and MMP-9 activity. Although the AhR activation mechanism still remains elusive, a reduction in pHi may be thought to be involved. These findings suggest a critical role for the AhR/c-Src axis in breast tumor progression stimulated by an acidic microenvironment.

KEYWORDS
aryl hydrocarbon receptor, breast cancer, c-Src, low pH, metalloprotease, migration

1 | INTRODUCTION

Tumor microenvironment, characterized by chronic inflammation, immune suppression, and hypoxia, influences the growth and evolution of tumor cells.[1] The increased glucose consumption and aerobic glycolysis results in the synthesis of lactic acid and $H+$ ions which must be controlled to prevent changes in intracellular pH (pHi), as pHi affects almost all cellular processes.[2] For this reason, cancer cells use different mechanisms to extrude protons, leading to the

acidification of the extracellular medium in the range of 6.5–6.9 that persists over time due to the poor vascular perfusion that characterize tumor tissues, impeding adequate proton clearance.[3–5] Acidification of pHe presents an advantage for cancer cells, increasing their proliferation and invasiveness.[6] Indeed, the major regions of tumor invasion correspond to areas with the lowest pHe, while noninvasion regions present normal pHe levels.[7] Although it is well known that extracellular acidosis contributes to tumorigenesis, the underlying mechanism of action still remains elusive.

Breast cancer is the most common tumor among women, ranking among the top five in terms of mortality.[8] The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor which is overexpressed in human mammary tumors[9,10] and shows greater activation in breast cancer cell lines with high malignancy.[11] The AhR is located in a cytosolic complex with c-Src kinase and, after activation, AhR can trigger nongenomic signaling events, including the release and activation of c-Src (AhR/c-Src axis), resulting in the phosphorylation of growth factor receptors.[12] AhR/c-Src activation induced by the AhR ligand hexachlorobenzene (HCB) promotes breast cancer progression, inducing cell migration and invasion as well as metalloproteases-2 (MMP-2) and -9 activity in MDA-MB-231 breast cancer cells.[13] Given that both the AhR/c-Src pathway and the slightly acidic tumor microenvironment promote breast cancer progression, we hypothesize that this signaling is activated by changes in the pHe. Our aim was to examine the effect of pHe 6.5 on AhR/c-Src signaling in MDA-MB-231 and LM3—two breast cancer cell lines—and NMuMG—a mammary epithelial cell line—as well as their association with cell migration and MMPs activities.

2 | MATERIALS AND METHODS

2.1 | Cell culture and treatment

The MDA-MB-231 cell line (American Type Culture Collection, ATCC) represents a highly aggressive and invasive triple-negative human breast cancer. The LM3 cell line was established from spontaneous murine mammary adenocarcinoma[14] and it is highly metastatic and epidermal growth factor receptor 2 (HER-2) positive. The nontumorigenic NMuMG cell line (ATCC) was derived from normal mammary gland tissue of an adult NAMRU mouse. The cell lines were cultured with Roswell Park Memorial Institute (RPMI)-1640 (HyClone Laboratories, Inc.) supplemented with 10% fetal bovine serum (FBS; Invitrogen Life Technology), 1% antibiotic–antimycotic mixture (10,000 Units/ml penicillin, 10 mg/ml streptomycin sulfate, and 25 mg/ml amphotericin B), and 1% glutamine (Sigma-Aldrich Chemical), and were maintained at 37°C in a humidified atmosphere supplemented with 5% CO_2.

For treatment, cells were seeded in 24-well plates in complete growth medium and 24 h later, cells were exposed to acidic medium (pH 6.5) or control medium (pH 7.4) supplemented with 5% FBS. Extracellular acidification was achieved by adding a precalculated volume of isotonic hydrogen chloride solution (Sigma-Aldrich Chemical), and pH was assessed by means of a pH meter (Hanna Instruments). In addition, MDA-MB-231 cells were exposed to 0.05 μM HCB (Aldrich-Chemie GmbH & Co.) for 15 min as a positive control of AhR/c-Src activation.[15] When indicated, cells were pretreated for 1 h with specific inhibitors: 5 μM 4,7-orthophenanthroline (PHE; Sigma-Aldrich Chemical) or 1 μM Stemregenin 1 (SR1; Cayman Chemical) for AhR, and 0.2 nM 4-Amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo(3,4-d)pyrimidine (PP2; Calbiochem) for c-Src. On the other hand, cells were incubated with 100 μM amiloride (Sigma-Aldrich Chemical) in the control medium (pH 7.4) supplemented with 5% FBS for 4 h to acidify intracellular pH (pHi).

2.2 | Gelatin zymography

Aliquots (20–50 μl) of cell-conditioned media were resuspended in Laemmli modified buffer, loaded on 6% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels containing 1% gelatin and electrophoresed as described by Pontillo et al.[13] MMP-2 and −9 activities were visualized through 0.5% Coomassie brilliant blue R-250 staining. Results were normalized to arbitrary units, designating a value of 1 to control assays.

2.3 | Measurement of intracellular ph (pHi)

Measurement of pHi was performed using BCECF-AM (2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, ace toxymethyl ester; Thermo Fisher) as Díaz et al.[16] described. MDA-MB-231 and LM3 cells (1×10^6/ml in RPMI) were loaded with 2 μg/ml BCECF-AM during 15 min at 37°C, and then washed and resuspended in RPMI 5% FBS adjusted to pH 7.4 or 6.5. Cells were incubated for 4 h at 37°C, and the values of pHi for each condition were determined. Flow cytometry analysis was performed, with excitation at 488 nm and emission analysis at FL1 and FL3. pHi was calculated from the ratio of emission intensities at the two wavelengths, standardizing by comparison with the fluorescence intensity ratios of cells whose pHi values were fixed by incubation with nigericin (10 μM) in high-potassium buffers.

2.4 | Migration assay

Scratch motility assay was performed as Miret et al.[17] described. The scratched area was photographed at 0 and 8 h for MDA-MB-231 and LM3, and at 0 and 18 h for NMuMG. The distance of wound healing in each well was evaluated and the migration rates were calculated as $D_{t0} - D_{tf}/(D_{t0} \times 100)$. D_{t0} = distance at 0 h and D_{tf} = distance at 8 h or 18 h, respectively.

2.5 | Western blot analysis

Total cell protein lysates (40 μg) was resolved by 7.5% SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore). The membranes were incubated with anti-P-c-Src (1:500) and then reblotted for c-Src (1:500) (Cell Signaling Technology) and for anti-β-actin (1:2000) antibodies (Sigma-Aldrich Chemical) overnight at 4°C, as previously described.[17]

2.6 | Statistical analysis

Student's *t* test with Welch's correction was used to identify significant differences between cells growing in the control medium (pHe 7.4) and the acidic medium (pHe 6.5). One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used to identify significant differences between the controls and treatments in time-course studies. Tukey post-hoc test was used to identify not only the action of the acidic media but also the effect of the inhibitor. The differences were considered significant when p values were ≤ 0.05. The results represent the mean ± *SD* of at least three independent experiments.

3 | RESULTS

3.1 | C-Src kinase activation in acidic ph medium

First, we evaluated whether acidic pHe promotes AhR/c-Src activation by analyzing c-Src phosphorylation in Tyrosine 416 (Y416), required for optimal activity.[18] Cells were grown in pHe 7.4 or pHe 6.5 medium for 30 min, 1, 2, 4, and 6 h, and c-Src activation was studied by Western blot. As shown in Figure 1A, acidosis significantly increased Y416-c-Src phosphorylation at 4 and 6 h of exposure in MDA-MB-231 cells. HCB exposure (positive control) also induced c-Src activation, as previously reported.[15] In LM3 cells, c-Src phosphorylation was enhanced after 4 h of treatment (Figure 1B). Nevertheless, the reduction in pHe was unable to activate c-Src in NMuMG cells (Figure 1C).

3.2 | Role of AhR and acidic pHi in c-Src activation

To establish the involvement of AhR in the previously enhanced c-Src phosphorylation, MDA-MB-231, and

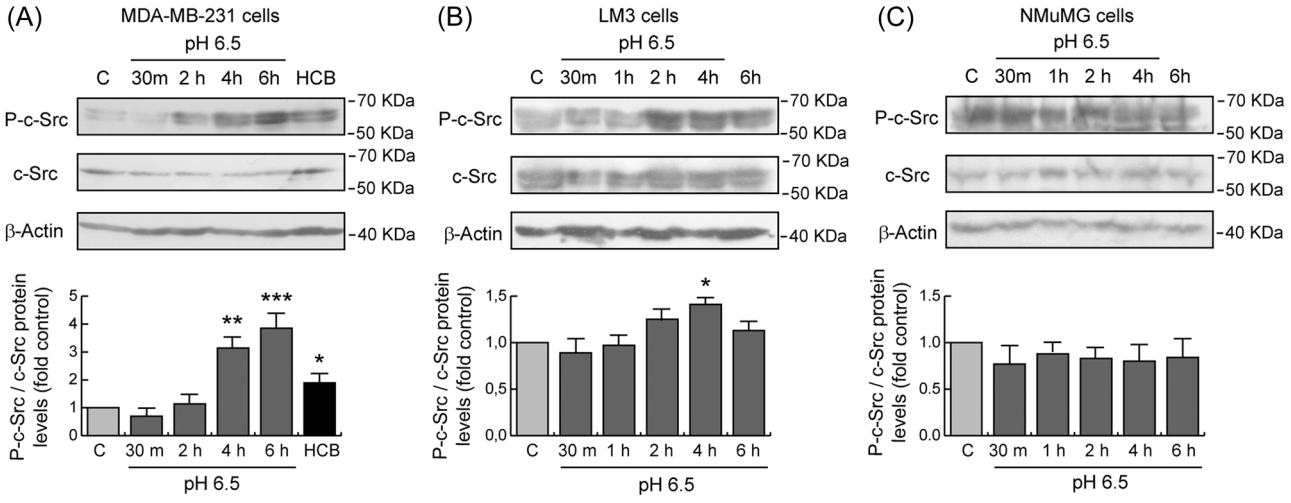

FIGURE 1 Acid pHe effects on AhR signaling pathways. Cells were exposed to acidic or control (C) medium and phospho (P)-c-Src and total c-Src protein levels in (A) MDA-MB-231, (B) LM3, and (C) NMuMG cells were analyzed by Western blot. Positive control: exposure to 0.05 μM HCB for 15 min. The corresponding P-Y416-c-Src/total c-Src ratio was normalized to control and quantification of at least three independent experiments is shown. Data are expressed as means ± *SD*. Asterisks indicate significant differences versus control (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ANOVA and Dunnett's post hoc test). ANOVA, analysis of variance.

LM3 cells were cultured in the presence or absence of AhR inhibitor PHE (5 μM). In addition, AhR inhibitor SR1 (1 μM) was used in MDA-MB-231, as it is known to block receptor activity only in human cell lines.[19] Results show that pretreatment with PHE or SR1 blocked acid-induced c-Src phosphorylation in breast cancer cells (Figure 2A,B,D).

This AhR/c-Src activation in the absence of ligand treatment may be explained by changes in pHi resulting from the reduction in pHe, which could alter the structure of the complex to which AhR and c-Src belong. To analyze this hypothesis, the BCECF-AM fluorescence probe was used to measure pHi in MDA-MB-231 and LM3 cell lines. Results showed a decrease in pHi after treatment with acidic medium from 7.60 ± 0.03 to 6.90 ± 0.02 ($p \leq 0.001$) in MDA-MB-231 and from 7.59 ± 0.02 to 7.27 ± 0.04 ($p \leq 0.05$) in LM3 cells.

To examine whether acidic pHi could be involved in AhR/c-Src activation, MDA-MB-231 and LM3 cells were treated with 100 μM amiloride in the control medium (pHe 7.4), which is known to reduce pHi by blocking the Na^+-H^+ exchanger 1 (NHE1).[20] Figure 2C,E shows that

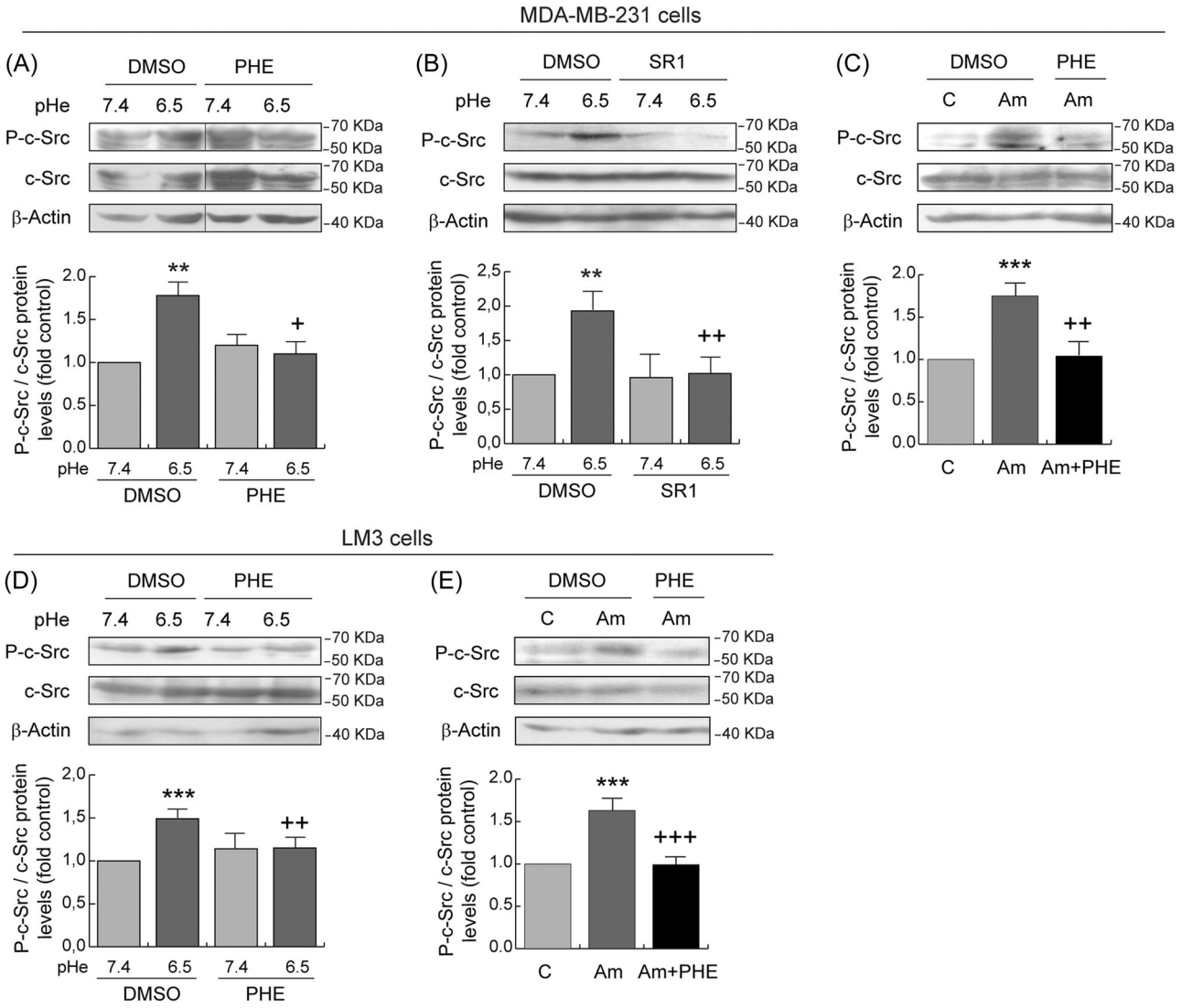

FIGURE 2 Role of AhR and NHE1 in c-Src phosphorylation. Phospho (P)-c-Src and total c-Src protein levels in (A-C) MDA-MB-231 and (D, E) LM3 cell lines. (A, B, D) Cells were pretreated with 5 μM PHE, 1 μM SR1, or vehicle (DMSO) and then exposed to acidic or control medium for 4 h, in the presence or absence of the inhibitor. (C, E) Cells were pretreated with PHE (5 μM) or vehicle (DMSO) and then cells were exposed to amiloride (Am, 100 μM) or vehicle (DMSO) for 4 h, in the presence or absence of the inhibitor. Quantification of p-Y416-c-Src/total c-Src ratio by densitometry scanning of the immunoblots is shown. Data are expressed as means ± *SD* of at least three independent experiments. Asterisks indicate significant differences versus control (*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$) and crosses indicate significant differences versus acidic pHe or amiloride (+$p \leq 0.05$; ++$p \leq 0.01$; +++$p \leq 0.001$). ANOVA and Tukey post hoc test. ANOVA, analysis of variance.

amiloride induced c-Src phosphorylation and that this effect was prevented by PHE.

3.3 | Effects of acidic pHe on cell migration

Extracellular acidification contributes to increasing tumor cell motility in lung,[21] prostate[1], and breast cancer.[22] Herein, wound healing assays in this study show that the acidic medium increased MDA-MB-231 and LM3 cell migration but had no effect on the mammary epithelial cell line NMuMG (Figure 3).

Considering that AhR signaling and c-Src kinase have been implicated in regulating cell motility,[23,24] we assessed their possible involvement in the effects of acidosis on MDA-MB-231 and LM3 cell migration. Cells were pretreated with the specific inhibitors, 5 μM PHE for AhR and 0.2 nM PP2 for c-Src, and then exposed to the acidic medium in the presence or absence of the inhibitors. Both inhibitors prevented MDA-MB-231 and LM3 wound closure (Figure 3A–D).

3.4 | Changes in MMP activity induced by acidic ph treatment

MMP-2 and −9 are closely associated with metastasis, as they degrade the basal membrane and thus allow tumor cell migration and invasion.[25] Gel zymography results showed that low pHe increased MMP-9 activity in MDA-MB-231 at 6 h (Figure 4A), as well as in LM3 cells at 6 and 24 h (Figure 4B), while no effects were observed on NMuMG cells (Figure 4C). MMP-2 activity showed no changes in cells growing in acidosis conditions (Figure 4A–C). To evaluate AhR and c-Src participation in acidosis-induced MMP-9 activity, cells were pretreated PHE or PP2 and then exposed to the acidic medium. Acidosis activated MMP-9 in an AhR- and c-Src-dependent manner in the MDA-MB-231 (Figure 4D). In LM3 cells, acidosis-induced MMP-9 activity was prevented by both inhibitors after 24 h of treatment, but only by PP2 after 6 h (Figure 4E), which hints at an AhR-independent mechanism after this time of exposure.

4 | DISCUSSION

Acidosis is a major tumor microenvironment factor present in most solid tumors, including breast cancer, and is typical of a malignant phenotype.[26,27] This study is consistent with previous results indicating that an acidic tumor microenvironment strongly influences breast cancer progression, as acidosis increases the MMP-9 proteolytic activity and boosts breast cancer cell migration.

The present investigation seeks to shed light on the mechanism of action responsible for these effects (Figure 5). Elevated c-Src activity has been implicated in cancer development, as it initiates signaling pathways that increase cell migration and invasion.[18] Our findings indicate that c-Src is a key factor for acidosis-induced cell motility in breast cancer, as pHe 6.5 promotes Y416-c-Src phosphorylation, which triggers migration and MMP-9 activity in MDA-MB-231 and LM3 cells. In agreement, pHe 6.6 also activates c-Src in hepatocellular carcinoma cells, which results in an expansion in cell migration and invasion.[28]

c-Src could be functionally attached to the AhR complex and activated upon ligand binding to AhR.[15,29,30] AhR plays an important role in tumor development[23] acting as a tumor suppressor or a tumorigenic factor, depending on the ligand, the AhR signaling pathways activated and the cellular context. For instance, the stimulation of nuclear AhR signaling is related to breast cancer prevention,[29,31] and the AhR ligands omeprazole and tranilast decrease MDA-MB-231 cell motility.[32,33] However, the AhR/c-Src axis activation promotes breast alterations and tumor progression[34,35] and the AhR agonist HCB and chlorpyrifos boost breast cancer cell migration and invasion.[13,15,36] Herein, AhR triggers acidosis-induced c-Src activation only in breast cancer cells and in the absence of exogenous ligands. This is a novel result, as the involvement of AhR in acidosis-induced breast cancer promotion had not been previously reported. Given that changes in pH alter the structure of c-Src regulatory domains,[37,38] a possible explanation for AhR/c-Src activation could be associated with the reduction in pHi observed after treatment with the acidic medium. This hypothesis is supported by the fact that amiloride exposure, which is known to reduce pHi,[20] also exerts c-Src phosphorylation in an AhR-dependent manner.

The acidic microenvironment promotes the remodeling of the extracellular matrix and increases tumor cell migration, invasion, and metastasis.[7,39,40] Our data show that pHe 6.5 induced cell migration through AhR and c-Src activation in breast cancer cells but had no effect on NMuMG cells. Similar results were obtained when MMP-9 activity was assayed in MDA-MB-231 cells; however, it is clear that different signaling pathways are being promoted in LM3 cells, as MMP-9 activation is AhR-dependent only at 24 h. An alternative mechanism could be related to the finding by Gupta et al.,[40] who observed that a reduction in pHe in breast cancer cells induces an enhancement in intracellular calcium content, increasing reactive oxygen species (ROS) levels which promotes cell invasion and metastasis. Likewise,

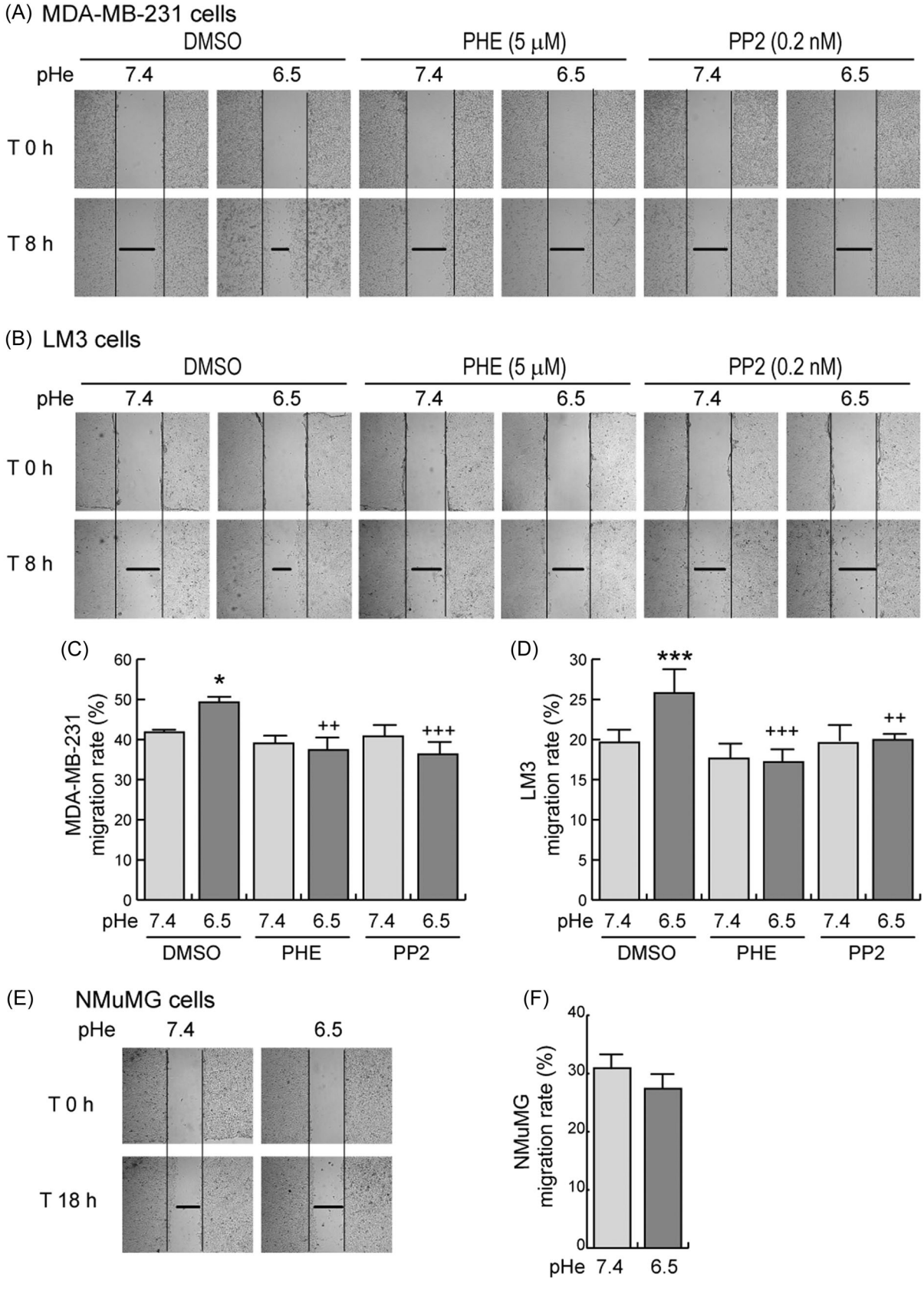

FIGURE 3 (See caption on next page)

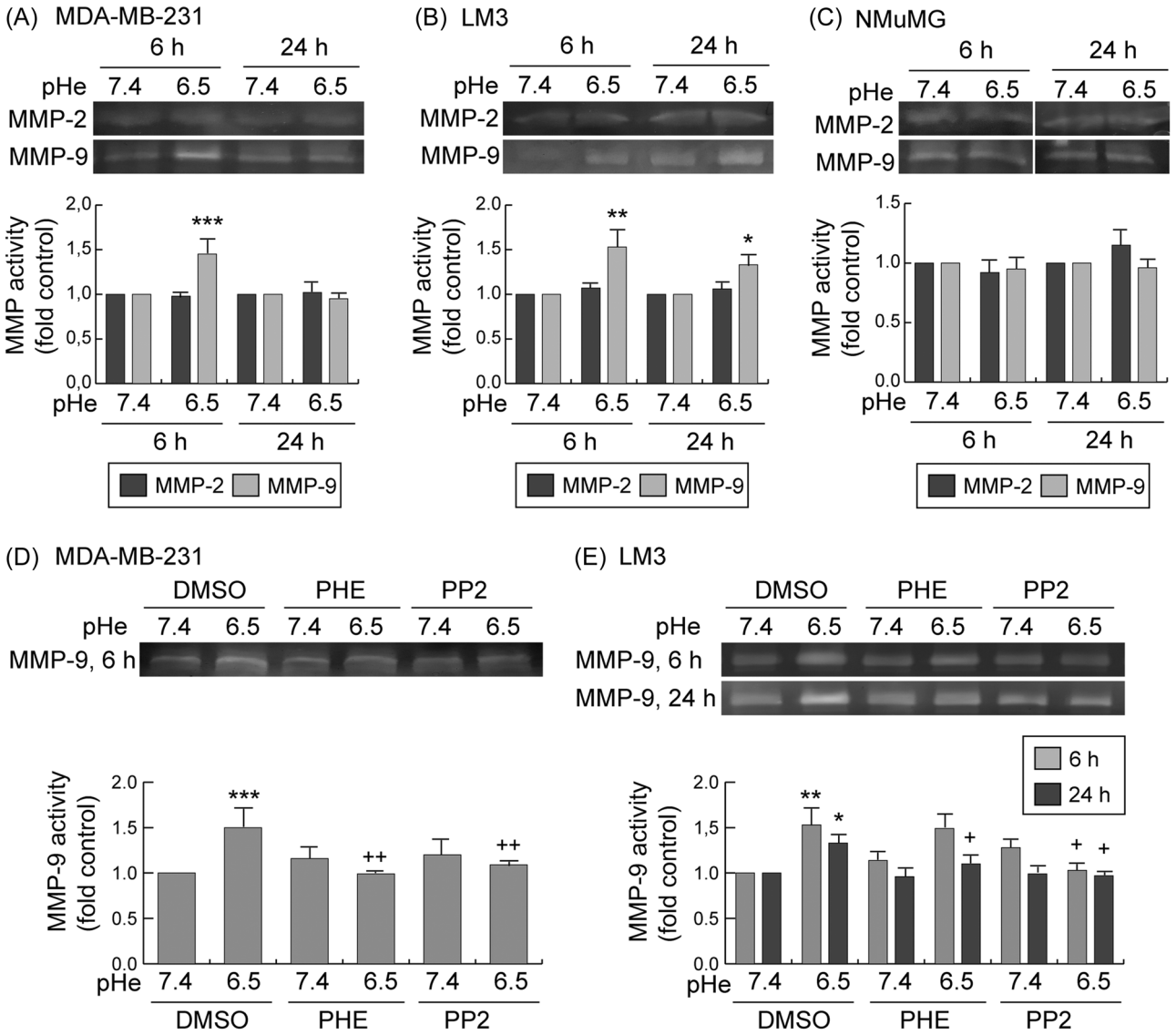

FIGURE 4 Acidosis effects on MMP activity. MMP-2 and MMP-9 activities in (A, D) MDA-MB-231, (B, E) LM3, and (C) NMuMG cell lines. (A–C) Cells were treated with acidic or control medium for 6 and 24 h, cell culture-conditioned media were collected and MMP-2 and MMP-9 activities were quantified by gelatin zymography. (D–E) Cells were pretreated with PHE (5 μM), PP2 (0.2 nM), or vehicle (DMSO) and then treated with acidic medium for (D–E) 6 or (E) 24 h, in the presence or absence of the inhibitor. Quantification by densitometric scanning of MMPs activities is shown. Data are expressed as means ± *SD* of at least three independent experiments. Asterisks indicate significant differences versus control (*$p \le 0.05$; **$p \le 0.01$; ***$p \le 0.001$) and crosses indicate significant differences versus acid medium (+$p \le 0.05$; ++$p \le 0.01$). (A–C) Student's *t* test with Welch's correction, (D,E) ANOVA and Tukey post hoc test. ANOVA, analysis of variance.

c-Src is activated through an increase in intracellular calcium[41] and ROS upregulation induces MMP-9 activity and invasion in breast cancer.[42] Additionally, radiotherapy generates redox imbalance, increasing ROS, which inhibit protein tyrosine phosphatases.[43] This leads to activation of receptor and nonreceptor tyrosine kinases as the epidermal growth factor receptor family, which often determines the resistance of cells to chemotherapy

FIGURE 3 Acidic pHe increases migratory ability in breast cancer cell lines. Wound-healing assay in (A) MDA-MB-231, (B) LM3, and (E) NMuMG cell lines. Cells were pretreated with specific inhibitors (5 μM PHE for AhR or 0.2 nM PP2 for c-Src) and then the monolayer was scratched with a pipette tip and treated with acidic pH (6.5) in the presence or absence of the inhibitors. The migration rate (%) of (C) MDA-MB-231, (D) LM3, or (F) NMuMG cells of at least three independent experiments were represented as mean ± *SD*. Asterisks indicate significant differences versus control (*$p \le 0.05$; ***$p \le 0.001$) and plus indicate significant differences versus acidic medium (++$p \le 0.01$; + ++$p \le 0.001$). ANOVA and Tukey post hoc test. ANOVA, analysis of variance.

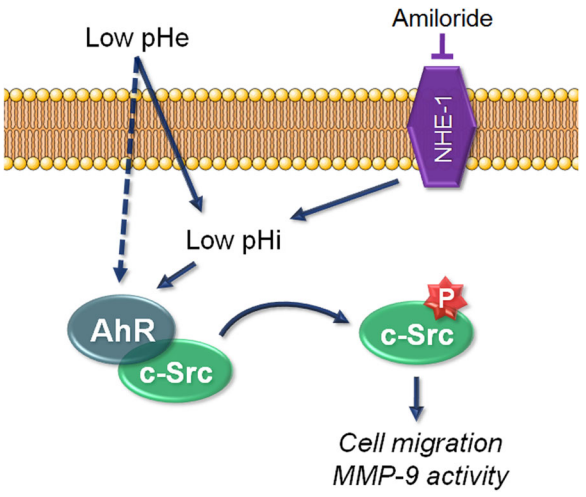

FIGURE 5 Model depicting how the acidic tumor microenvironment promotes cell migration and MMP-9 activity through the AhR/c-Src signaling pathway. pHe 6.5 induces a reduction in pHi in breast cancer cells, activating AhR/c-Src axis and increasing c-Src phosphorylation. Similar results are observed after amiloride treatment. Then, c-Src activation triggers cell migration and MMP-9 proteolytic activity. We cannot rule out the possibility that there are other mechanisms by which this pathway is activated (dotted arrow). MMP-9, metalloprotease 9.AhR, aryl hydrocarbon receptor. NHE-1, Na +-H^+ exchanger 1.

or radiotherapy.[44,45] Among the main activated pathways by these receptors, is that mediated by c-Src.[46] Herein, we showed that low pHe activates c-Src and thus increases cell motility, suggesting that extracellular acidosis activates pathways that favor radio and chemoresistance.

Moreover, the proteolytic activity of MMP-9[47,48] and MMP-2[49] is markedly increased at low pH in melanoma and prostate cancer. However, in this study, only MMP-9 was modulated by pHe reduction, without changes in MMP-2 activity in any cellular type. To persist, cancer cells must be able to adapt to an acidic tumoral environment, which also pushes neoplastic cells toward a more aggressive phenotype.[27] In contrast, this environment is toxic to normal cells and this is probably the reason why reduced pH showed no effects on cell migration or MMPs activity in breast epithelial cells.

In summary, this study demonstrates that extracellular acidosis induces a reduction in pHi in breast cancer cells, activating the AhR/c-Src axis. This results in c-Src phosphorylation, triggering cell migration and MMP-9 proteolytic activity. These findings suggest a critical role for the AhR/c-Src axis in breast tumor progression stimulated by an acidic microenvironment. In the next step, it would be relevant to carry out studies in animals or in 3D models of tumor cells that could contribute to validate our present results obtained in vitro. It is essential to understand how acidosis confers advantages to tumor cells, since the characterization of the signaling pathways involved may path the way to discover new therapeutic targets.

AUTHOR CONTRIBUTIONS

A. Randi and J. Geffner conceived, designed, and supervised the study. N.V. Miret and L.V. Zárate developed the cell cultures assays. F. Erra Díaz performed the pHi determination and collaborated with the amiloride assays. N.V. Miret, L.V. Zárate, M.A. Leguizamón, and C.A. Pontillo carried out the western blot and zymography tests. F.A. Chiappini and L. Ceballos collaborated analyzing the migration results blinded to the treatments. N.V. Miret and A.S. Randi analyzed the results and wrote the manuscript, while L.V. Zárate and J. Geffner reviewed it.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Riemann A, Schneider B, Gundel D, Stock C, Gekle M, Thews O. Acidosis promotes metastasis formation by enhancing tumor cell motility. *Adv Exp Med Biol*. 2016;876:215-220. doi:10.1007/978-1-4939-3023-4_27
2. Putnam RW. Intracellular pH regulation. In: Sperelakis N., ed. *Cell physiology sourcebook*. 4th ed. Academic Press; 2012: 357-372.
3. Gatenby RA, Gawlinski ET, Gmitro AF, Kaylor B, Gillies RJ. Acid-mediated tumor invasion: a multidisciplinary study. *Cancer Res*. 2006;66:5216-5223. doi:10.1158/0008-5472.CAN-05-4193
4. Koltai T. Cancer: fundamentals behind pH targeting and the double-edged approach. *Onco Targets Ther*. 2016;9:6343-6360. doi:10.2147/OTT.S115438

5. Webb BA, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. *Nat Rev Cancer*. 2011;11:671-677. doi:10.1038/nrc3110
6. Liberti MV, Locasale JW. The Warburg effect: how does it benefit cancer cells? *Trends Biochem Sci*. 2016;41(3):211-218. doi:10.1016/j.tibs.2015.12.001
7. Estrella V, Chen T, Lloyd M, et al. Acidity generated by the tumor microenvironment drives local invasion. *Cancer Res*. 2013;73:1524-1535. doi:10.1158/0008-5472.CAN-12-2796
8. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424. doi:10.3322/caac.21492
9. Mohamed HT, Gadalla R, El-Husseiny N, et al. Inflammatory breast cancer: Activation of the aryl hydrocarbon receptor and its target CYP1B1 correlates closely with Wnt5a/b-β-catenin signalling, the stem cell phenotype and disease progression. *J Adv Res*. 2019;16:75-86. doi:10.1016/j.jare.2018.11.006
10. Powell JB, Goode GD, Eltom SE. The aryl hydrocarbon receptor: a target for breast cancer therapy. *J. Cancer Ther*. 2013;4(7):1177-1186. doi:10.4236/jct.2013.47137
11. Eltom SE, Gasmelseed A, Saoudi-Guentri D. The aryl hydrocarbon receptor is over-expressed and constitutively activated in advanced breast carcinoma. *Cancer Res*. 2006;66(8):408.
12. Dong B, Cheng W, Li W, et al. FRET analysis of protein tyrosine kinase c-Src activation mediated via aryl hydrocarbon receptor. *Biochim Biophys Acta*. 2011;1810(4):427-431. doi:10.1016/j.bbagen.2010.11.007
13. Pontillo C, Rojas P, Chiappini F, et al. Action of hexachlorobenzene on tumor growth and metastasis in different experimental models. *Toxicol Appl Pharmacol*. 2013;268:331-342. doi:10.1016/j.taap.2013.02.007
14. Urtreger A, Ladeda V, Puricelli L, et al. Modulation of fibronectin expression and proteolytic activity associated with the invasive and metastatic phenotype in two murine mammary cell lines. *Int J Oncol*. 1997;11:489-496. doi:10.3892/ijo.11.3.489
15. Pontillo C, García MA, Peña D, et al. Activation of c-Src/HER1/STAT5b and HER1/ERK1/2 signaling pathways and cell migration by hexachlorobenzene in MDA-MB-231 human breast cancer cell line. *Toxicol Sci*. 2011;120:284-296. doi:10.1093/toxsci/kfq390
16. Díaz FE, Dantas E, Cabrera M, et al. Fever-range hyperthermia improves the anti-apoptotic effect induced by low pH on human neutrophils promoting a proangiogenic profile. *J. Cell Death Dis*. 2016;7(10):e2437. doi:10.1038/cddis.2016.337
17. Miret N, Pontillo C, Ventura C, et al. Hexachlorobenzene modulates the crosstalk between the aryl hydrocarbon receptor and transforming growth factor-β1 signaling, enhancing human breast cancer cell migration and invasion. *Toxicology*. 2016;366:20-31. doi:10.1016/j.tox.2016.08.007
18. Frame MC. Src in cancer: deregulation and consequences for cell behaviour. *Biochim Biophys Acta*. 2002;1602(2):114-130. doi:10.1016/s0304-419x(02)00040-9
19. Boitano AE, Wang J, Romeo R, et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science*. 2010;329(5997):1345-1348. doi:10.1126/science.1191536
20. Kim GE, Lyons JC, Song CW. Effects of amiloride on intracellular pH and thermosensitivity. *Int J Radiat Oncol Biol Phys*. 1991;20(3):541-549. doi:10.1016/0360-3016(91)90067-e
21. Wu Y, Gao B, Xiong QJ, Wang YC, Huang DK, Wu WN. Acid-sensing ion channels contribute to the effect of extracellular acidosis on proliferation and migration of A549 cells. *Tumour Biol*. 2017;39(6):1010428317705750. doi:10.1177/1010428317705750
22. Nakanishi M, Korechika A, Yamakawa H, Kawabe N, Nakai K, Muragaki Y. Acidic microenvironment induction of interleukin-8 expression and matrix metalloproteinase-2/-9 activation via acid-sensing ion channel 1 promotes breast cancer cell progression. *Oncol Rep*. 2021;45:1284-1294. doi:10.3892/or.2020.7907
23. Barouki R, Coumoul X, Fernandez-Salguero PM. The aryl hydrocarbon receptor, more than a xenobiotic-interacting protein. *FEBS Lett*. 2007;581:3608-3615. doi:10.1016/j.febslet.2007.03.046
24. Bourguignon LY, Wong G, Earle C, Krueger K, Spevak CC. Hyaluronan-CD44 interaction promotes c-Src-mediated twist signaling, microRNA-10b expression, and RhoA/RhoC up-regulation, leading to Rho-kinase-associated cytoskeleton activation and breast tumor cell invasion. *J Biol Chem*. 2010;285(47):36721-36735. doi:10.1074/jbc.M110.162305
25. Li H, Qiu Z, Li F, Wang C. The relationship between MMP-2 and MMP-9 expression levels with breast cancer incidence and prognosis. *Oncol Lett*. 2017;14:5865-5870. doi:10.3892/ol.2017.6924
26. Ibrahim-Hashim A, Estrella V. Acidosis and cancer: from mechanism to neutralization. *Cancer Metastasis Re.* 2019;38:149-155. doi:10.1007/s10555-019-09787-4
27. Sadeghi M, Ordway B, Rafiei I, et al. Integrative analysis of breast cancer cells reveals an epithelial-mesenchymal transition role in adaptation to acidic microenvironment. *Front Oncol*. 2020;10:304. doi:10.3389/fonc.2020.00304
28. Chen KH, Tung PY, Wu JC, et al. An acidic extracellular pH induces Src kinase-dependent loss of beta-catenin from the adherens junction. *Cancer Lett*. 2008;267:37-48. doi:10.1016/j.canlet.2008.03.005
29. Baker JR, Sakoff JA, McCluskey A. The aryl hydrocarbon receptor (AhR) as a breast cancer drug target. *Med Res Rev*. 2020;40:972-1001. doi:10.1002/med.21645
30. Park S, Dong B, Matsumura F. Rapid activation of c-src kinase by dioxin is mediated by the Cdc37-HSP90 complex as part of ah receptor signaling in MCF10A cells. *Biochemistry*. 2007;46:899-908. doi:10.1021/bi061925f
31. Hall JM. The aryl-hydrocarbon receptor (AhR) as a therapeutic target in human breast cancer. *J. Steroids Horm. Sci*. 2014;5:3. doi:10.4172/2157-7536.1000140
32. Jin UH, Lee SO, Pfent C, Safe S. The aryl hydrocarbon receptor ligand omeprazole inhibits breast cancer cell invasion and metastasis. *BMC Cancer*. 2014;14:498. doi:10.1186/1471-2407-14-498
33. Subramaniam V, Ace O, Prud'homme GJ, Jothy S. Tranilast treatment decreases cell growth, migration and inhibits colony formation of human breast cancer cells. *Exp Mol Pathol*. 2011;90(1):116-122. doi:10.1016/j.yexmp.2010.10.012
34. Miret NV, Pontillo CA, Zárate LV, Kleiman de Pisarev D, Cocca C, Randi AS. Impact of endocrine disruptor

hexachlorobenzene on the mammary gland and breast cancer: the story thus far. *Environ Res*. 2019;173:330-341.
35. Ye M, Zhang Y, Gao H, et al. Activation of the aryl hydrocarbon receptor leads to resistance to EGFR TKIs in non-small cell lung cancer by activating Src-mediated bypass signaling. *Clin Cancer Res*. 2018;24:1227-1239. doi:10.1158/1078-0432.CCR-17-0396
36. Lasagna M, Hielpos MS, Ventura C, et al. Chlorpyrifos subthreshold exposure induces epithelial-mesenchymal transition in breast cancer cells. *Ecotoxicol Environ Safety*. 2020;205:111312. doi:10.1016/j.ecoenv.2020.111312
37. Bacarizo J, Martinez-Rodriguez S, Martin-Garcia JM, et al. Electrostatic effects in the folding of the SH3 domain of the c-Src tyrosine kinase: pH-dependence in 3D-domain swapping and amyloid formation. *PLoS One*. 2014;9(12):e113224. doi:10.1371/journal.pone.0113224
38. Réty S, Fütterer K, Grucza RA, Munoz CM, Frazier WA, Waksman G. pH-Dependent self-association of the Src homology 2 (SH2) domain of the Src homologous and collagen-like (SHC) protein. *Protein Sci*. 1996;5(3):405-413. doi:10.1002/pro.5560050301
39. Boczek EE, Luo Q, Dehling M, et al. Autophosphorylation activates c-Src kinase through global structural rearrangements. *J Biol Chem*. 2019;294(35):13186-13197. doi:10.1074/jbc.RA119.008199
40. Gupta SC, Singh R, Asters M, et al. Regulation of breast tumorigenesis through acid sensors. *Oncogene*. 2016;35:4102-4111. doi:10.1038/onc.2015.477
41. Dong B, Matsumura F. Roles of cytosolic phospholipase A2 and Src kinase in the early action of 2,3,7,8-tetrachlorodibenzo-p-dioxin through a nongenomic pathway in MCF10A cells. *Mol Pharmacol*. 2008;74:255-263. doi:10.1124/mol.107.044669
42. Mori K, Uchida T, Yoshie T, et al. A mitochondrial ROS pathway controls matrix metalloproteinase 9 levels and invasive properties in RAS-activated cancer cells. *FEBS J*. 2019;286:459-478. doi:10.1111/febs.14671
43. Chiarugi P, Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci*. 2003;28(9):509-514. doi:10.1016/S0968-0004(03)00174-9
44. Nyati MK, Morgan MA, Feng FY, Lawrence TS. Integration of EGFR inhibitors with radiochemotherapy. *Nat Rev Cancer*. 2006;6(11):876-885. doi:10.1038/nrc1953
45. Schmidt-Ullrich RK, Mikkelsen RB, Dent P, et al. Radiation-induced proliferation of the human A431 squamous carcinoma cells is dependent on EGFR tyrosine phosphorylation. *Oncogene*. 1997;15(10):1191-1197. doi:10.1038/sj.onc.1201275
46. Ishizawar R, Parsons SJ. c-Src and cooperating partners in human cancer. *Cancer Cell*. 2004;6(3):209-214. doi:10.1016/j.ccr.2004.09.001
47. Huang S, Tang Y, Peng X, et al. Acidic extracellular pH promotes prostate cancer bone metastasis by enhancing PC-3 stem cell characteristics, cell invasiveness and VEGF-induced vasculogenesis of BM-EPCs. *Oncol Rep*. 2016;36(4):2025-2032. doi:10.3892/or.2016.4997
48. Peppicelli S, Bianchini F, Torre E, Calorini L. Contribution of acidic melanoma cells undergoing epithelial-to-mesenchymal transition to aggressiveness of non-acidic melanoma cells. *Clin Exp Metastasis*. 2014;31(4):423-433. doi:10.1007/s10585-014-9637-6
49. Rofstad EK, Mathiesen B, Kindem K, Galappathi K. Acidic extracellular pH promotes experimental metastasis of human melanoma cells in athymic nude mice. *Cancer Res*. 2006;66(13):6699-6707. doi:10.1158/0008-5472.CAN-06-0983

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