Integrative Bioinformatics Analysis Reveals Potential Target Genes and TNFα Signaling Inhibition by Brazilin in Metastatic Breast Cancer Cells

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Abstract

Objective: Metastasis is the most significant cause of morbidity and mortality in breast cancer patients. Previously, a combination of brazilin and doxorubicin has been shown to inhibit metastasis in HER2-positive breast cancer cells. This present study used an integrative bioinformatics approach to identify new targets and the molecular mechanism of brazilin in inhibiting metastasis in breast cancer. Methods: Cytotoxicity and mRNA arrays data were retrieved from the DTP website, whereas genes that regulate metastatic breast cancer cells were retrieved from PubMed with keywords “breast cancer metastasis”. Gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, and Drug association analysis were carried out by using WEB-based GEne SeT AnaLysis Toolkit (WebGestalt). Construction of protein-protein interaction (PPI) network analysis was performed by STRING-DB v11.0 and Cytoscape, respectively. The genetic alterations of the potential therapeutic target genes of brazilin (PB) were analyzed using cBioPortal. Results: Analysis of cytotoxicity with the public database of COMPARE showed that brazilin exerts almost the same cytotoxicity in the NCI-60 cells panel showing by similar GI50 value, in which the lowest GI50 value was observed in MDA-MB 231, a metastatic breast cancer cells. KEGG enrichment indicated several pathways regulated by brazilin such as TNF signaling pathway, cellular senescence, and pathways in cancer. We found ten drugs that are associated with PB, including protein kinase inhibitors, TNFα inhibitors, enzyme inhibitors, and anti-inflammatory agents. Conclusion: In conclusion, this study identified eight PB, including MMP14, PTGS2, ADAM17, PTEN, CCL2, PIK3CB, MAP3K8, and CXCL3. In addition, brazilin possibly inhibits metastatic breast cancer through inhibition of TNFα signaling. The study results study need to be validated with in vitro and in vivo studies to strengthen scientific evidence of the use of brazilin in breast cancer metastasis inhibition.

Keywords: Metastasis- breast cancer- bioinformatics- TNFα signaling- Brazilin

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molecular targets and their molecular changes of brazilin in metastatic breast cancer cells needs to be done.

This present study used an integrative bioinformatics approach to identify new targets and molecular mechanisms of brazilin in inhibiting metastases in breast cancer. The brazilin target was retrieved from the NCI COMPARE, while the metastatic breast cancer regulatory gene was downloaded from PubMed, which from both we made a Venn diagram consisting of the potential therapeutic target of brazilin against metastatic breast cancer cells (Figure 1). Analysis of protein-protein interaction network, gene ontology, enrichment of the KEGG pathway, and genetic alterations, reveal the targets and molecular mechanisms of brazilin in inhibiting metastatic breast cancer. This present study could be the basis for the development of brazilin as an antimetastatic agent in breast cancer.

Materials and Methods

Data mining and processing

Cytotoxic effect and mRNA expression data were collected from the NCI 60 DTP website (http.dtp.nci.nih.gov) (Monks et al., 1997). COMPARE analysis with the public library generates a list of high similarity drugs with brazilin, and a list of mRNA expressions upon brazilin treatment in the NCI 60 cells collection (Mahmoud et al., 2018). The similarity pattern is presented as the Pearson correlation coefficient, with the cutoff value of <-0.3 and > 0.3. Regulatory genes of metastatic breast cancer were retrieved from PubMed database with keywords “breast cancer metastasis”.

Gene ontology and KEGG pathway enrichment analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were carried out by using Overrepresentation Enrichment Analysis (ORA) from WEB-based GEne Set AnaLysis Toolkit (WebGestalt), (Wang et al., 2017), with p<0.05 as the cutoff value.

Drug association analysis

Drug association analysis was performed using Overrepresentation Enrichment Analysis (ORA) from WEB-based GEne Set AnaLysis Toolkit (WebGestalt) with p<0.05 and FDR<0.05 was selected as the cutoff value (Wang et al., 2017).

PPI network and hub genes analysis

PPI network was analyzed with STRING-DB v11.0 (Szklarczyk et al., 2015), with confidence scores >0.7 were considered significant. PPI network further was visualized by Cytoscape. Selection of hub genes based on the highest degree score was conducted by CytoHubba plugin (Chin et al., 2014).

Analysis of genetic alterations of the PB

The genetic alterations of the potential therapeutic target genes of brazilin (PB) were analyzed using cBioPortal (Cerami et al., 2012; Gao et al., 2013). The breast cancer study with the highest genetic alterations was chosen for further connectivity analysis with cutoff value of p < 0.05.

Results

Data mining and analysis

This present study aimed to identify the new molecular targets and mechanism of brazilin in inhibition of metastatic breast cancer cells. Analysis of cytotoxicity with the public database of COMPARE showed that brazilin exerts almost the same cytotoxicity in the NCI-60 cells panel showing a similar GI50 value (Figure 2A). The lowest GI50 value was observed in prostate and breast cancer. Moreover, the GI50 value of brazilin in MDA-MB 231, metastatic breast cancer cells, was one of the lowest amongst other cell lines (Supplementary Table 1).

Analysis with COMPARE identified 13 standard agents with a correlation with brazilin, either direct or inverse (Supplementary Table 2). Pancratistatin and S-trityl-L-cysteine are standard drugs with the highest score of the correlation coefficient, whereas AT-125 (acivicin) and mitomide are standard drugs with the lowest score of Pearson correlation coefficient (Supplementary Table 2). COMPARE analysis revealed 1249 genes regulated by brazilin (Supplementary Table 3), including 587 and 662 genes with the positive and negative Pearson correlation coefficient, respectively. GLRX3, SMG5, SLC6A4 (with Pearson correlation coefficient of 0.476, 0.459, 0.455, respectively) are genes with direct correlation. SLC7A11, IL37, and PHKB, with a Pearson correlation coefficient of -0.592, -0.551, and -0.547, respectively, are genes with inverse correlation. Direct correlation shows that the higher mRNA expression, the higher the chemoresistance, while inverse correlation shows that the higher expression of mRNA, the higher the chemosensitivity of the drugs.

Since brazilin exerted high cytotoxicity towards metastatic breast cancer cells, we retrieved 2,263 regulatory genes of metastasis in breast cancer (Figure 2B). Moreover, a Venn diagram between Brazilin target from COMPARE and regulatory genes of metastatic breast cancer, revealed 102 genes, which were then considered as potential targets of brazilin in metastatic breast cancer (PB).

GO, KEGG pathway, and drug association analysis of potential targets of brazilin in metastatic breast cancer

Gene ontology analysis was categorized into biological process, cellular component, and molecular function (Figure 2C). We found that PB was mostly involved in response to stimulus, metabolic process, and cell communication. In addition, the PB was located in the membrane, nucleus, and cytosol, and play a role in the molecular function in protein, ion and nucleic acid binding, as well as enzyme regulator activity. KEGG enrichment indicated several pathways regulated by brazilin (Table 2) such as the TNF signaling pathway, cellular senescence, and pathways in cancer. Several PB was involved in TNFα signaling pathway, including CASP3, CCL2, CXCL3, MAP3K8, MMP14, PIK3CB, PTGS2, and TNFRSF1A (Supplementary Table 5, Figure 3). From drug association analysis, we found ten drugs
Integrative Bioinformatics Analysis of Brazilianin (22%) (Figure 5B).

Discussion

This study was aimed to identify the new targets and molecular mechanisms of brazilin in inhibition of metastases in breast cancer using an integrative bioinformatics approach. Analysis using COMPARE showed that brazilin had the lowest GI50 value amongst other cells in MDA-MB 231 cells, highly metastatic breast cancer cells (Liu et al., 2019). This phenomenon revealed the high potency of brazilin as an anticancer agent against metastatic breast cancer.

Analysis with COMPARE identified 13 standard agents that have a correlation with brazilin, in which pancratistatin, S-trityl-L-cysteine are standard drugs with the highest score of Pearson correlation coefficient, whereas AT-125 (acivicin) and mitindomide are standard drugs with the lowest score of Pearson correlation coefficient. The high value of the Pearson correlation coefficient means, the higher the cytotoxicity of a standard compound, the higher the cytotoxicity of brazilin. Vice versa, the low value of the Pearson correlation coefficient means the higher the cytotoxicity of a standard compound, the lower the cytotoxicity of brazilin. A previous study showed that pancratistatin, that are associated with brazilin, including protein kinase inhibitors, TNFα inhibitors, enzyme inhibitors, and anti-inflammatory agents (Table 2).

PPI network and hub genes analysis

A PPI network were constructed from 102 proteins (confidence level of 0.4) consists of 99 nodes, 233 edges, PPI enrichment value of <1.10e-16, and average local clustering coefficient of 0.396) (Figure 4A). The top 20 of highest degree score genes were revealed, including, PTEN, CASP3, PTGS2, and ADAM17 (Figure 4B, Table 3).

Analysis of genetic alterations of the PB

Eight potential target genes of brazilin (PB) were analyzed using cBioportal to explore their genomic alterations across breast cancer studies. CCL2, MAP3K8, MMP14, PIK3CB, and CXCL3 were selected from KEGG pathway enrichment analysis. PTGS2, MMP14, ADAM17, PTEN, CCL2, and CXCL3 were selected based on the highest degree score using CytoHubba. A study, namely the METABRIC (Lefebvre et al., 2016), was selected for further analysis (Figure 5A). Genetic alterations for each target genes were found as MMP14 (1.2%), ADAM17 (1.2%), PIK3CB (1.6%), MAP3K8 (1.6%), CXCL3 (1.9%), CCL2 (2%), PTEN (7%), and PTGS2 (22%) (Figure 5B).

Table 1. KEGG Pathway Enrichment Analysis of the DEGs

| Gene Set | Description | P Value | FDR       |
|----------|-------------|---------|-----------|
| hsa04668 | TNF signaling pathway | 1.02E-07 | 0.000033174 |
| hsa04218 | Cellular senescence | 0.000025109 | 0.0025615 |
| hsa04060 | Cytokine-cytokine receptor interaction | 0.000026574 | 0.0025615 |
| hsa04657 | IL-17 signaling pathway | 0.000033405 | 0.0025615 |
| hsa05200 | Pathways in cancer | 0.000039287 | 0.0025615 |
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications | 0.000050078 | 0.0027209 |
| hsa05418 | Fluid shear stress and atherosclerosis | 0.000059126 | 0.0027536 |
| hsa05220 | Chronic myeloid leukemia | 0.000096584 | 0.003861 |
| hsa05206 | MicroRNAs in cancer | 0.00010659 | 0.003861 |
| hsa05163 | Human cytomegalovirus infection | 0.00034585 | 0.011275 |
| hsa05142 | Chagas disease (American trypanosomiasis) | 0.00048594 | 0.014402 |
| hsa04659 | Th17 cell differentiation | 0.00062734 | 0.014837 |
| hsa04931 | Insulin resistance | 0.00062734 | 0.014837 |
| hsa04932 | Non-alcoholic fatty liver disease (NAFLD) | 0.00063716 | 0.014837 |
| hsa04115 | p53 signaling pathway | 0.00069172 | 0.015033 |
| hsa04390 | Hippo signaling pathway | 0.00077566 | 0.015802 |
| hsa04217 | Necroptosis | 0.0010456 | 0.018937 |
| hsa04630 | JAK-STAT signaling pathway | 0.0010456 | 0.018937 |
| hsa04380 | Osteoclast differentiation | 0.0015995 | 0.027443 |
| hsa04068 | FoxO signaling pathway | 0.0018718 | 0.030511 |
| hsa05222 | Small cell lung cancer | 0.0020866 | 0.032393 |
| hsa04010 | MAPK signaling pathway | 0.0023689 | 0.035103 |
| hsa05215 | Prostate cancer | 0.0026322 | 0.037308 |
| hsa05161 | Hepatitis B | 0.002903 | 0.039432 |
| hsa04625 | C-type lectin receptor signaling pathway | 0.0035609 | 0.046434 |
| hsa05230 | Central carbon metabolism in cancer | 0.0037962 | 0.047599 |

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Integrative Bioinformatics Analysis of Brazilin

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Analysis with COMPARE identified 13 standard agents that have a correlation with brazilin, in which pancratistatin and S-trityl-L-cysteine are standard drugs with the highest score of Pearson correlation coefficient, whereas AT-125 (acivicin) and mitindomide are standard drugs with the lowest score of Pearson correlation coefficient. The high value of the Pearson correlation coefficient means, the higher the cytotoxicity of a standard compound, the higher the cytotoxicity of brazilin. Vice versa, the low value of the Pearson correlation coefficient means the higher the cytotoxicity of a standard compound, the lower the cytotoxicity of brazilin. A previous study showed that pancratistatin,
alkaloid compound isolated from Amaryllidaceae plant, promotes apoptosis and autophagy in DU145 and LNCaP metastatic prostate cancer cells (Griffin et al., 2011). Another study demonstrated that S-trityl-L-cysteine isolated from garlic is a novel Eg5 inhibitor for chemotherapy against neuroblastoma cells (Wu et al., 2018). Acivicin, an antibiotic, and chemotherapy isolated from Streptomyces sviceus (Poster et al., 1981). A phase II clinical trial of acivicin has been performed against advanced metastatic breast cancer patients (Fleishman et al., 1983). Moreover, a combination of acivicin and cisplatin inhibits metastasis in B16F10 melanoma cells (Roy and Maity, 2007). Another study showed that mitindomide exerts cytotoxicity through inhibition of topoisomerase II (Hasinoff et al., 1997). Accordingly, brazilin probably acts as pancriastatin in inhibiting metastatic breast cancer cells.

KEGG pathway enrichment analysis revealed that brazilin targets the TNF signaling pathway in inhibition of metastatic breast cancer cells. Tumor necrosis factor-alpha (TNFα) is a cytokine that regulates various biological processes of cancer, including inflammation (Scheff et al., 2017), cell proliferation and apoptosis (Lyu et al., 2017), progression and metastasis (Ham et al., 2016), and chemoresistance (Zhang et al., 2018). The signaling starts when TNFα binds into its receptor, TNFαRI (a member of TNFαR family together with TNFαRII), and leads to receptor trimerization and the recruitment of adaptor protein and TNFαR associated factor, followed by activation of IKK and transactivation of NFkB (Wu and Zhou, 2010).

TNFα signaling pathway regulates metastasis in breast cancer through several mechanisms. Activation of NFkB by TNFα promotes breast cancer by promoting proliferation and survival, inhibiting apoptosis, and inducing angiogenesis. Moreover, TNFα can activate NFkB through various mechanisms, including direct binding of TNFαRI to NFkB, activation of mitogen-activated protein kinases (MAPKs), and activation of JAK-STAT signaling pathway.

Table 2. Top 10 Drug Association Analysis

| Gene Set | Description                                | P value   | FDR        |
|----------|--------------------------------------------|-----------|------------|
| PA164712838 | Interleukin inhibitors                   | 6.63E-11  | 1.21E-07  |
| PA164713204 | Protein kinase inhibitors                | 3.57E-09  | 0.000003264 |
| PA164713366 | Tumor necrosis factor alpha (TNF-alpha) inhibitors | 7.16E-08  | 0.000037631 |
| PA166049190 | flufenamic acid                          | 8.24E-08  | 0.000037631 |
| PA164712732 | Enzyme inhibitors                        | 1.08E-07  | 0.000039341 |
| PA164712734 | Enzymes                                  | 1.71E-07  | 0.000051958 |
| PA164712839 | Interleukins                             | 1.76E-06  | 0.00045974  |
| PA164774763 | latanoprost                              | 0.000016719 | 0.0038181 |
| PA164712458 | Antiinflammatory Agents                  | 0.000021643 | 0.0043934 |
| PA450744   | Oxygen                                    | 0.00027381 | 0.0044111 |

Table 3. Top 20 Hub Genes Based on Highest Score Degree, Analyzed by CytoHubba

| Rank | Gene Symbol | Gene Name                              | Score |
|------|-------------|----------------------------------------|-------|
| 1    | PTEN        | Phosphatase and tensin homolog         | 26    |
| 2    | CASP3       | Caspase-3                              | 25    |
| 3    | PTGS2       | Prostaglandin G/H synthase 2           | 19    |
| 3    | CCL2        | C-C motif chemokine 2                  | 19    |
| 5    | LEP         | Leptin                                 | 16    |
| 6    | IL17A       | Interleukin-17A                        | 15    |
| 7    | SMAD3       | Mothers against decapentaplegic homolog 3 | 14    |
| 8    | TNFRSF1A    | Tumor necrosis factor receptor superfamily member 1A | 13 |
| 8    | SOCS3       | Suppressor of cytokine signaling 3     | 13    |
| 10   | FOXP3       | Forkhead box protein P3                | 12    |
| 10   | MMP1        | Interstitial collagenase               | 12    |
| 10   | CDKN2A      | Cyclin-dependent kinase inhibitor 2A   | 12    |
| 13   | CDH5        | Cadherin-5                             | 10    |
| 13   | SELE        | E-selectin                             | 10    |
| 15   | HSP90AB1    | Heat shock protein HSP 90-beta         | 9     |
| 15   | TGFBR1      | TGF-beta receptor type-1              | 9     |
| 15   | MDM2        | E3 ubiquitin-protein ligase Mdm2       | 9     |
| 15   | MMP14       | Matrix metalloproteinase-14           | 9     |
| 15   | YWHAZ       | 14-3-3 protein zeta/delta             | 9     |
| 20   | ADAM17      | Disintegrin and metalloproteinase domain-containing protein 17 | 8 |
cell proliferation and migration in breast cancer cells (Kawabata et al., 2017). TNFα signaling also activates the ERK/JNK/p38 pathway leading to migration and invasion in colon cancer cells (Zhao and Zhang, 2018), and breast cancer cells (Qiu et al., 2019). Another study demonstrated that TNFα activates mesenchymal stromal cells that leads to metastasis in breast cancer through the recruitment of CXCR2+ neutrophils (Yu et al., 2017). In addition, TNFα contributes to the aggressive properties of triple-negative breast cancer cell lines via the upregulation of TNFAIP3(A20) (Lee et al., 2019). Recently, TNFα involves in the invasion and metastasis of breast cancer cells by promoting crosstalk between mitochondria and lysosomal function (Singh et al., 2019). The results of KEGG pathway enrichment analysis is supported by the results of drug association analysis, which showed that potential therapeutic target of brazilin against metastatic breast cancer cells (PB) are associated with tyrosine kinase inhibitors and TNFα inhibitors (Supplementary Table 6). Nevertheless, these findings need to be validated further with in vitro and in vivo studies.

In the following section, we will discuss each gene target, its potential as a target of brazilin, and its relationship to TNFα signaling. MMP14 encodes matrix metalloproteinase 14 (Mmp14) or also known as membrane type-1 matrix metalloproteinase (MT1-MMP), an enzyme that plays a role in the breakdown of extracellular matrix in invasion, metastasis, and angiogenesis (Shuman Moss et al., 2012). Overexpression of MMP14 in serum was found in patients with invasive gastric cancer (Kasurinen et al., 2018). MMP19 plays a role in the TNFα signaling pathway. TNFα increased the expression of MMP14 during fracture healing (Lehmann et al., 2005). Moreover, MMP19 plays a role in the TNFα signaling pathway. TNFα increased the expression of MMP14 during fracture healing (Lehmann et al., 2005). MMP19 plays a role in the TNFα signaling pathway.
subsequently promotes the migration of breast cancer cells through the upregulation of MMP9, MMP14, and MMP2 in the lipid rafts (Wolczyk et al., 2016). Nevertheless, the role of MMP14 and TNFα signaling upon brazilin treatment needs to be explored further. 

PTGS2 encodes prostaglandin-endoperoxide synthase 2.
synthase 2 or known as cyclooxygenase 2 (COX2), a key regulatory enzyme in the biosynthesis of prostaglandin E2 (Kozak et al., 2000). Overexpression of COX2 was found in about 40% of patients with invasive breast cancers (Singh et al., 2006). COX2 promotes bone metastasis of breast cancer cells through the upregulation of Interleukin-11 (IL-11) (Singh et al., 2006). Previous studies showed that COX2 promotes metastasis in breast cancer cells through several mechanisms, including infiltration by regulatory T cells (Tregs) (Karavitis and Zhang, 2013) and activation of the VEGF signaling pathway to trigger angiogenesis (Xu et al., 2014).

The crosstalk between COX2 and TNFα was discussed in several previous studies. A previous study showed that TNFα induces the COX2 expression via activation of the NFkB signaling pathway in human colon cancer cells (Plummer et al., 1999), and activation of P38/MAPK pathway in colonic myofibroblast (Saini et al., 2016). TNFα also promotes migration in colonic myofibroblast via activation of COX2 that is mediated by the P38/MAPK pathway (Saini et al., 2016). Moreover, TNFα also induces COX2 expression and subsequently increased PGE2 production and MMP9 expression in human fibroblast-like synovial cells (Alsousi et al., 2017).

ADAM17 encodes ADAM metallopeptidase domain 17, a member of a disintegrin and metalloprotease domain family, and is also known as tumor necrosis factor (TNF)-converting enzyme (Li et al., 2019c). A previous study demonstrated that ADAM17 plays a role in the shedding of TNFα and membrane receptors protein, which involved in the biological process of proliferation, migration, and differentiation (Gutiérrez-López et al., 2011). On the other hand, ADAM17 is a pivotal enzyme for the generation of TNF-α in oral keratinocytes (Hirayama et al., 2017), and hemophilic arthropathy (Haxaire et al., 2018). Overexpression of ADAM17 promotes 5-fluorouracil resistance and metastasis in colorectal cancer cells via activation of the Notch signaling pathway.

Figure 5. (A). Overview of genetic changes in MMP14, PTGS2, ADAM17, PTEN, CCL2, PIK3CB, MAP3K8, and CXCL3 across 16 breast cancer studies, as analyzed by cBioportal. (B). Summary of alterations in MMP14, PTGS2, ADAM17, PTEN, CCL2, PIK3CB, MAP3K8, and CXCL3 across breast cancer patients using a study from Pereira et al., (2016).
pathway (Li et al., 2018), and induces metastasis in gastric cancer through activation of Notch and Wnt signaling pathways (Li et al., 2019c). A review article discussed ADAM17 inhibitors for inflammation and cancer therapy (Kanda et al., 2017). However, the role of brazilin in ADAM17 activity and TNFα in promoting metastasis need to be clarified further.

PTEN encodes a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase, a negative regulator of PI3K/Akt signaling pathway (Maehama et al., 2001). A previous study revealed the association between the loss of PTEN and metastasis in patients with endometrial cancer (Salvesen et al., 2002), prostate and breast cancer cells (Bandopadhuy et al., 2004). Previous studies revealed the role and mechanisms of PTEN in breast cancer metastasis. PTEN inhibits proliferation, migration, and invasion in osteosarcoma cells by downregulating MMP9 and focal adhesion kinase (FAK) (Hu et al., 2014). Another study demonstrated that PTEN hampers invasion and metastasis of gastric cancer cells through the inhibition of the PI3K/NFκB pathway and preventing the DNA binding of NFκB on the FAK promoter (Zhang et al., 2014). Moreover, PTEN inhibits metastasis in breast cancer cells through the downregulation of WNT1 inducible signaling protein 1 (WISP1) and lipocalin-2 (LCN2) (Chiang et al., 2016), and inactivation of NFκB signaling in non-small cell lung cancer cells (Akgun et al., 2019). A previous study showed that TNFα increased the expression of PTEN through activation of NFκB signaling in HL60 human leukemic cells (Lee et al., 2007). However, other studies demonstrated that PTEN inhibited the PI3K/AKT/NFκB signaling pathway that is induced by TNFα in human glioma cells (Koul et al., 2006), and prostate cancer cells (Lee et al., 2007). Further study of brazilin on the axis of PTEN-NFκB-TNFα in metastatic breast cancer cells is required.

The results of this present study showed genetic alterations in 7% of patients with metastatic breast cancer in the METABRIC study (Figure 4B). This result is supported by a recent study which demonstrated that mutation in PTEN leads to inactivation of its function as tumor suppressor genes in cancer (Luongo et al., 2019). A recent review article discussed the possibility of PTEN as a potential biomarker of lymph node metastasis of esophageal squamous cell carcinoma (Li et al., 2019b). Taken together, PTEN plays a pivotal role in breast cancer metastasis.

CCL2 encodes C-C motif chemokine ligand 2, also known as monocyte chemoattractant protein 1 (MCP1), a chemokine that regulates inflammatory processes (Daly and Rollins, 2003). Recent studies showed that CCL2 also regulates cancer metastasis. A previous study showed that CCL2 induces chemokine cascade signaling and subsequently promotes breast cancer metastasis by elevating retention of metastasis-associated macrophages (Kitamura et al., 2015). Another study demonstrated that CCL2 plays a role in macrophage recruitment that regulates lymphatic metastasis of bladder cancer (Chen et al., 2018). Increased CCL2 secretion was also found in the protumoral microenvironment induced by retinoblastoma inactivation (Li et al., 2019a).

The axis between TNFα signaling and CCL2 has also been studied, in which TNFα increases the expression of CCL2 in human proximal tubular epithelial cells via activation of MAPK signaling (Ho et al., 2008). A recent clinical trial of an inhibitor of CCL2, namely propagermanium, showed proper safety and efficacy in patients with metastatic breast cancer (Masuda et al., 2020). Taken together, CCL2 is a promising target for inhibiting breast cancer metastasis; however, the role of brazilin in metastasis-related to CCL2 and TNFα signaling needs to be explored further.

PIK3CB encodes phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta (PI3Kβ), also known as p105, a protein involved in the PI3K/Akt signaling pathway (Pridham et al., 2018). Overexpression of PIK3CB was found in patients with colorectal carcinoma (Wen et al., 2014), non-small cell lung cancer (Xiong et al., 2017). Inhibition of PIK3CB with a specific inhibitor, reduced cell viability, and proliferation in glioblastoma (Pridham et al., 2018). In addition, PIK3CB, a significant regulator of the PI3K/Akt pathway, regulates metastasis in breast cancer cells (Hong et al., 2019). The PI3K/Akt signaling is involved in the TNFα pathway in airway remodeling (Jude et al., 2012). Activation of PI3K/Akt signaling increased the secretion of TNFα in activating macrophages (Huang et al., 2013). Nevertheless, the effects of brazilin on PIK3CB in metastatic breast cancer cells remain unclear.

MAP3K8 encodes mitogen-activated protein kinase kinase kinase 8, a member of serine/threonine kinase family (Paardekooper et al., 2018), which is involved in the MAPK/JNK/p38 and NFκB signaling pathway (Chorzalska et al., 2018). Overexpression of MAP3K8, also known as tumor progression locus 2 (TPL2), is correlated with poor prognosis and metastasis in patients with colorectal cancer (Pyo et al., 2018). Another study demonstrated that MAP3K8 induces invasion and metastasis in renal cancer cells (Liu et al., 2016). MAP3K8 was also found to involve in the maintenance of thyroid cancer stem cells and thyroid cancer resistance to vemurafenib (Giani et al., 2019). Genetic alterations in MAP3K8, including fusion and truncation, were found in 33% of patients with Spitz melanoma (Newman et al., 2019). Regarding the crosstalk with TNFα signaling, MAP3K8 was found to increase the expression of TNFα in monocyte-derived dendritic cells (Paardekooper et al., 2018). Accordingly, the role of MAP3K8 in metastatic breast cancer and the effect of brazilin on MAP3K8 need to be explored in the future study.

CXCL3 encodes C-X-C motif chemokine ligand 3, a member of the CXC subfamily of chemokines, also known as a growth-related oncogene, is a potent neutrophil chemoattractant that regulates inflammation (Smith et al., 2005). The upregulation of CXCL3 was found in metastatic breast cancer and possesses a potential therapeutic target (See et al., 2014). Other studies showed that CXCL3 is involved in migration and invasion of trophoblasts in the pathogenesis of preeclampsia (Wang et al., 2018) and is involved in the proliferation and migration of prostate cancer cells (Xin et al., 2018). A previous study demonstrated that CXCL3 is upregulated upon treatment.
of TNFα in adipocytes (Kusuyama et al., 2016). In addition, TNFα induced the upregulation of CXCL3 and its receptor in A498 renal cancer cells (Sun et al., 2016). Taken together, CXCL3 and its signaling is a potential target for preventing metastatic breast cancer. However, the effect of brazilin on CXCL3 signaling related to TNFα in metastatic breast cancer remains elusive.

Previous studies have demonstrated the effect of brazilin on TNFα signaling and PB. Brazilin suppressed the production of TNFα in lipopolysaccharide-induced RAW264.7 macrophages cells (Hu et al., 2009). Brazilin inhibited TNFα-induced NFKB signaling by targeting IKK in 293/IL-1R/TLR4 cells (Jeon et al., 2014). Brazilin was shown to decrease high glucose-induced vascular inflammatory through the inhibition of NFκB activation in HUVEC cells (Jayakumar et al., 2014). Brazilin was also found to decrease the expression of TNFα in mice with type-II collagen-induced arthritis (Jung et al., 2015) and to decrease the expression of COX2 and TNFα, and inhibit ERK/NFKB signaling in RANKL-stimulated RAW264.7 cells (Kim et al., 2015). Recently, a study showed that brazilin possesses anti-inflammatory activity in TNFα-induced psoriasis dermatitis by downregulating ERK/JNK/p38 and NFKB signaling (Choi and Hwang, 2019). Further study of the effects brazilin on TNFα signaling and PB in metastatic breast cancer cells is required.

This present study revealed the new target genes and the mechanism of brazilin in inhibiting metastasis in breast cancer using a bioinformatics approach. This study identified eight new potential targets of brazilin for inhibiting metastatic breast cancer, including MMP14, PTGS2, ADAM17, PTEN, CCL2, PIK3CB, MAP3K8, and CXCL3. In addition, brazilin possibly inhibits metastatic breast cancer through inhibition of the TNFα signaling. The study results need to be validated with in vitro and in vivo studies to strengthen scientific evidence of the use of brazilin in inhibition of breast cancer metastasis.

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Author contribution

AH-conception and design of the study, acquisition, analysis and interpretation of data, drafting and revising the article and final approval of the version to be published, HP-acquisition and analysis of data, drafting the article and final approval of the version to be published.

Availability of material

The datasets analysed during the present study are online available in the public database.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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