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Zerumbone: A Potent Emerging Phytochemical with Anticancer Therapeutic Potential

(Zerumbon: Kemunculan Fitokimia Poten dengan Potensi Terapeutik Antikanser)

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ABSTRACT

Breast cancer is a prevalent cause of global mortality, characterised by abnormal cell growth within the breast. These cells can spread to distant sites in the body through metastasis and one of the mechanisms that breast cancer cells use to metastasise is via invadopodia formation. Accumulated evidence has explained pathways that may contribute to the breast cancer cells metastasis including the ERK, SMAD-3, STAT3 and NF-κB pathways. The hypoxic conditions within tumours enhance their metastatic ability through HIF-1α upregulation. Despite advanced treatments including chemotherapy and radiotherapy, these approaches are expensive and sometimes lack efficacy. Zerumbone, a compound extracted from *Zingiber zerumbet*, is known for its anti-cancer properties. It counteracts cancer cell metastasis by reducing cell migration, invasion, and proliferation by acting upon multiple signalling pathways. This review recapitulates the metastasis of breast cancer and its biomarkers. In addition, our review will also explore the impact of zerumbone, therapeutic roles and its mechanism of action in reducing breast cancer metastasis.

Keywords: Breast cancer; hypoxia; invadopodia; metastasis; zerumbone

ABSTRAK

Kanser payudara adalah penyebab lazim kematian global, dicirikan oleh pertumbuhan sel yang tidak normal dalam payudara. Sel-sel ini boleh merebak ke tapak yang jauh dalam badan manusia melalui metastasis dan salah satu mekanisme yang digunakan oleh sel kanser payudara untuk metastasis adalah melalui pembentukan invadopodia. Bukti terkumpul telah menjelaskan mekanisme yang mungkin menyumbang kepada metastasis sel kanser payudara termasuk laluan yang melibatkan ERK, SMAD-3, STAT3 dan NF-κB. Keadaan hipoksik dalam tumor meningkatkan keupayaan metastatik mereka melalui peningkatan HIF-1α. Walaupun dengan kehadiran rawatan lanjutan termasuk kemoterapi dan radioterapi, rawatan ini memerlukan kos yang tinggi dan kadangkala kurang berkesan. Zerumbon, sebatian yang diekstrak daripada *Zingiber zerumbet*, terkenal dengan sifat anti-kansernya. Ia menentang metastasis sel kanser dengan mengurangkan penghijrahan, pencerobohan dan percambahan sel dengan bertindak melalui pelbagai mekanisme. Kajian ini menyusun semula metastasis kanser payudara dan biopenandanya. Di samping itu, penyelidikan kami juga akan mengkaji kesan zerumbon, peranan pemulihan dan mekanisme tindakannya dalam mengurangkan metastasis kanser payudara.

Kata kunci: Hipoksia; invadopodia; kanser payudara; metastasis; zerumbon

Introduction

According to the World Health Organisation (WHO), 2.3 million women worldwide were diagnosed with breast cancer in 2020 and the mortality rate was 685,000. There are four types of breast cancer which are ductal carcinoma *in situ*, invasive ductal carcinoma, inflammatory breast cancer and metastatic breast cancer, where metastatic breast cancer is thought to be the most incurable. Metastatic triple-negative breast cancer (TNBC) is characterised by the absence of oestrogen and progesterone receptors as well as a lack of human epidermal growth factor receptor (HER2) expression

(Yin et al. 2020). It is classified as a specific subtype of epithelial breast tumour and is the most aggressive form of breast cancer (O'Reilly et al. 2015; Yin et al. 2020). However, most therapies are expensive and sometimes exhibit limited efficacy, whereas anti-cancer drugs may lack selectivity (Castaneda & Strasser 2017). Conversely, plant-based products have been utilised as medicine ever since ancient days as phytochemicals have been thought to exert therapeutic effects against many human ailments. The major focus of pharmacological research is the identification of bioactive compounds and their underlying molecular mechanisms (Rajabi et al. 2021). One such compound that possesses anti-cancer properties is zerumbone, a sesquiterpenoid isolated from the rhizomes of *Zingiber zerumbet* (Girisa et al. 2019).

METASTATIC CASCADE

The metastatic cascade is a highly complex process whereby aggressive cancer cells leave the primary site where the tumour is formed and travel through the bloodstream to reach distant sites and develop into secondary tumours. The heterogenicity of metastasis has made cancer difficult to treat. The overall process of metastasis can be divided into three main stages which are invasion, intravasation and extravasation. The mechanism is mainly initiated by the local invasion of the cancer cells to the basement membrane and surrounding tissues. Particularly, the Epithelial-Mesenchymal Transition (EMT), which involves the loss of adherence junctions and apical-based interactions, as well as the acquisition of the mesenchymal phenotype leads to invasion (Lambert, Pattabiraman & Weinberg 2017). To metastasise, the tumour cells escape from the primary site which occurs due to continuous defects in chromosomal segregation and instability. Furthermore, a group of molecular mediators known as the cadherin family is involved in cell-to-cell adhesion. Specifically, E-cadherin, which primarily helps to maintain the integrity of epithelial cells are downregulated, promoting cancer cell invasion (Padmanaban et al. 2019).

Invasion is followed by the proteolytic degradation of extracellular matrix (ECM) which allows the penetration of cancer cells across tissue boundaries. As the cell junctions disrupt, the circulating tumour cells (CTCs) will move through the basement membrane into the blood vessels or lymphatic system, causing distant metastasis. Other cells, signalling molecules and proteases which are present in the tumour microenvironment (TME) affect intravasation as well. Immune cells, platelets, fibroblasts and adipocytes contribute to intravasation through cell-to-cell signalling, which results in TME alteration.

MIGRATION AND INVASION OF BREAST CANCER CELLS

Prior to migration, cells must undergo polarization. The attachment of cancer cells to ECM components such as fibronectin (FN), vitronectin, collagen, and fibrinogen is facilitated by integrins, which are transmembrane receptors (Scully et al. 2012). Actin reorganization leads to the formation of protrusions from breast cancer cells that aid in migration and invasion from the ECM to the bloodstream (Dattachoudhury et al. 2020). Besides that, the metastatic potential of breast cancer cells is governed by various other mechanisms. For instance, an overexpression of matrix metalloproteinases (MMPs) is often associated with breast cancer metastasis (Guo et al. 2020). Moreover, the NF-κB pathway significantly helps in the production of Interleukins (IL) and activation of epithelial-to-mesenchymal transition transcription factors (EMT-TFs) such as SNAIL, SLUG, SIP1 and TWIST1. Additionally, NF-κB aids in metastasis, tumour growth, angiogenesis, inflammation, and cell survival via transcriptional regulation of a wide range of genes (Sarkar et al. 2013).

INVADOPODIA FORMATION

Invadopodia are actin-rich protrusions that form finger-like projections which are capable of degrading the extracellular matrix (ECM), and their formation is involved in tumour metastasis. By facilitating the degradation of the ECM, invadopodia enable cancer cells to spread from their primary tumor site to other sites as illustrated in Figure 1. The process of tumor invasion includes three stages: invadopodia formation, invadopodia elongation, and maturation. Invadopodia play a significant role in organizing the metastatic cascade and rely on certain proteins, including Arp 2/3, cortactin, and various MMPs such as MMP-2, MMP-9, and MMP-14, to facilitate cancer cell migration and metastasis (Karamanou et al. 2020). One of their core structures, cortactin, is essential in promoting the nucleation of actin (Meirson & Gil-Henn 2018). On top of that, cortactin helps to stimulate the secretion of MMPs which promote the migration and invasion of cancer cells (Jeannot & Besson 2020). Peculiar proteolytic activity at the surface of invadopodia is capable of activating various types of growth factors that can contribute towards tumour growth and favour the process of angiogenesis (Karamanou et al. 2020). Invadopodia are thought to be the hallmark of tumour cells that undergoes metastasis which drives oncogenic mutations in the cells and produces new

tumour at the distant site (Jeannot & Besson 2020). Hence, this finding is considered an essential discovery in the molecular and cellular basis of tumour invasion (Om Alblazi & Siar 2015).

HYPOXIA AND TUMOUR MICROENVIRONMENT (TME)

Tumour can be defined as a mass of connective tissues with abnormal cells embedded in it, which contribute to the formation of a TME that is essential for tumorigenesis (Casey et al. 2015; Li, Zhao & Li 2021). Both oxygen and nutrients are necessary for malignant cells to continuously proliferate and survive. These cells tend to grow faster than their blood vessels formation. Hence, the effective oxygen diffusion rate of capillaries around the tumour cells cannot meet the needs of their rapid growth. This results in an uneven supply of oxygen in the tumour tissue thereby forming a hypoxic environment. Hypoxia has been widely studied for its strong influence towards cancer metastasis. It is one of the leading characteristics of solid tumours including breast cancer, and it provides a strong pressure for the most aggressive forms of tumour which contributes to an increased risk of tumour metastasis (Semenza 2001). Once the desired hypoxic microenvironment is established, the host immune system will downregulate all sorts of anti-tumour immune responses through a variety of mechanisms (Whiteside 2008). These mechanisms are either directly mediated by tumour-producing factors, or indirectly happen due to significant changes in normal tissue homeostasis which occur because of cancer.

REGULATION OF HYPOXIA-INDUCIBLE FACTOR-1-ALPHA (HIF-1α) IN BREAST CANCER

HIF-1α acts as a key regulator for tumour hypoxia, which affects tumour growth and metastasis in many cancer types (Eddy et al. 2017). These cancer cells increase the activity of HIF-1α in response to a reduction in oxygen supply. Along with HIF-1α overexpression, cancer cells may survive the adverse effects of hypoxic stresses via a variety of mechanisms such as changes in extensive gene expression through either activation or inactivation of certain genes (Hamad et al. 2019). It also helps to promote angiogenesis by inducing both HIF-1α and vascular endothelial growth factor (VEGF) expression. Previous studies have shown a significant increase of VEGF expression in TNBC compared to non TNBC (Linderholm et al. 2009; O'Reilly et al. 2015). An overexpression of HIF-1α triggers EMT through various proteins regulation such as Jagged2 and urokinase receptor (uPAR), promoting cancer cell invasion (Liu, Semenza & Zhang 2015). Consequently, the initiation of EMT leads to migration, invasion and metastasis. Not only does this contribute towards the development of chemoresistance phenotype of breast cancer cells, a hypoxic tumour environment is also responsible in inducing an immunosuppressive microenvironment in breast tumours (Hashimoto & Shibasaki 2015).

ZERUMBONE

Plant-derived natural products, best known as phytochemicals have been utilised by ancient civilizations to improve human health. Having said that, Zerumbone is a phytochemical isolated from *Z. zerumbet*, a type of ginger which is more commonly known as shampoo ginger or pinecone ginger (Kiyama 2020). Centuries ago, the rhizomes of *Z. zerumbet* have been used as a traditional medicine to treat health problems such as fever, asthma, allergies and wound care (Prasannan et al. 2012). Several studies have also shown that zerumbone can exert various pharmacological properties including antioxidative, anti-inflammatory, anti-microbial and anti-ulcer properties as summarized in Table 1. These properties could be associated with the presence of α,β-unsaturated carbonyl based moiety in its structure as shown in Figure 2 (Salminen et al. 2008). In addition, existing studies have elucidated the importance of zerumbone in cancer progression, in spite of the unclear mechanisms (Prasannan et al. 2012). This phytochemical has exhibited significant anti-cancer properties against different types of cancer including breast cancer (Woźniak et al. 2021). Having said that, various research has been conducted to determine the underlying molecular pathways along with the associated molecular targets of zerumbone against breast cancer.

MECHANISM OF ACTION OF ZERUMBONE AGAINST BREAST CANCER METASTASIS UNDER NORMOXIA

Zerumbone modulates CD1d-mediated anti-tumor response in breast cancer

Cancer cells modulate both innate and adaptive immune systems, which involve immune cells such as macrophages and natural killer cells, also known as antigen-presenting cells. The interaction of the natural killer cells receptors with a lipid antigen-presenting molecule called CD1d acts upon tumour cells by releasing perforin, granzymes, and cytokines, promoting the activation of other immune cells (Nair & Dhodapkar 2017). Although the role of CD1d in breast cancer is yet to be elucidated, this ligand apparently is expressed on variety cancer cells including breast cancer cells. Evidence shows the correlation between

the upregulation of CD1d and the progress of metastatic breast cancer. In the presence of zerumbone, essential components of the antigen-presenting pathway regulated by CD1d were significantly decreased as illustrated in Figure 3. Furthermore, zerumbone attenuates the CD1d expression by inhibiting the ERK1/2 pathway which inhibits cell proliferation (Shyanti et al. 2017). In another study, an anti-CD1d mAb was utilised with zerumbone to investigate its synergistic anti-cancer effects. This approach resulted in cell death which was mediated by the loss of mitochondrial membrane potential and apoptosis (Rizvi, Puri & Saxena 2015). This finding suggests the role of zerumbone as a potential anti-cancer treatment.

Zerumbone inhibits transforming growth factor-β (TGF-β)-induced signalling pathway

High expression of TGF-β is often associated with more aggressive forms of cancer, including TNBC (Tan, Alexe & Reiss 2009). TGF-β is regarded as the inducer of metastasis as it promotes the induction of FN, a large adhesive glycoprotein and various MMPs such as MMP-2 and MMP-9 (Moses & Barcellos-Hoff 2011). Also, TGF-β induces SMAD-3 phosphorylation in breast cancer, which modulates the expression of FN and associated MMPs as described in Figure 4. Additionally, SMAD-3 acts as a metastatic modulator and favours cell survival. Therefore, zerumbone has been used to directly act upon SMAD-3 and attenuate breast cancer progression. It was found that the treatment of breast cancer cells with zerumbone has shown suppression of SMAD-3 phosphorylation (Khoshakhlagh et al. 2019). In this case, after zerumbone treatment, the expression levels of FN, MMP-2 and MMP-9 have decreased, which in turn reduced the rate of cell migration. Thus, this explains the role of TGF-β-mediated SMAD-3 pathway and the potential of zerumbone to be used as a drug to suppress cell motility (Kim et al. 2015).

Zerumbone suppresses CD44 expression through STAT3 inhibition

The transcriptional activity of CD44 is mainly regulated by the epidermal growth factor receptor (EGFR) dependent pathway and its overexpression is often associated with an enhancement of metastasis. On top of that, an overexpressed EGFR is commonly seen with poor prognosis in breast cancer cells (Kim et al. 2014). Moreover, the phosphorylation of STAT3 elevates when breast cancer cells are treated with EGF. Activation of CD44 was decreased upon the use of STAT3 inhibitors, indicating that the phosphorylation of STAT3 plays an important role in inducing CD44 activation (Kim et al. 2014). Zerumbone exerts its anti-cancer effect by inhibiting the expression of STAT3 which then further suppresses the CD44 activation. It was reported that the basal mRNA expression levels of CD44 were decreased as well. Consistent with this report, the downregulation of CD44 may alter the rate of tumour progression. The expressions of EGF and TGF-α which normally act as an inducer of CD44 were also decreased, resulting in CD44 downregulation (Kim et al. 2014; Sung et al. 2008).

Zerumbone modulates the expression of interleukin 1 beta (IL-1β) via nuclear factor kappa B (NF-κB) pathway

Interleukins are pro-inflammatory cytokines produced by macrophages and monocytes. Interleukin 1 beta (IL-1β) in particular, is highly expressed in various types of cancer cells, including breast cancer cells (Elaraj et al. 2006). Aberrant IL-1β expression contributes to the relapse and metastasis of breast cancer (Jeon et al. 2016). A former study has shown that the cell invasion rate significantly increased when treated with IL-1β, whereas the use of IL-1β receptor antagonist decreased the rate of cell invasion (Han et al. 2014). IL-1β expression in breast cancer is modulated by transcription factors including NF-κB, whereby the overexpression of NF-κB increases the levels of IL-1β. Furthermore, the expression of IL-1β is usually higher in TNBC compared to non TNBC cells (Jeon et al. 2016). Nevertheless, zerumbone inhibits the phosphorylation of NF-κB and thus reduces the basal level of IL-1β, which typically triggers the NF-κB signalling pathway. However, zerumbone does not affect the IL-1-induced phosphorylation of the AKT pathway. This suggests that zerumbone has the ability to regulate the expression of IL-1β through the NF-κB pathway (Jeon et al. 2016).

Zerumbone effects on the CXC chemokine ligand 12 (CXCL12) expression

A chemokine, CXCL12 and its receptor called CXC chemokine receptor 4 (CXCR4) are overexpressed in many types of tumours, leading to a poor prognosis. In breast cancer, the abnormal activation of HER2 induces the CXCR4 expression in breast cancer cells, resulting in metastasis (Li et al. 2004). Another study discovered the induction of CXCR4 expression when breast cancer cells are cultured under hypoxic conditions (Schioppa et al. 2003). Despite the reason for its overexpression being unclear, it has been established that the association

between this protein and cancer biology occurs via two distinct mechanisms. These mechanisms involve direct autocrine effects and indirect effects on CXCL12 (Guo et al. 2016). As for the direct effects, this chemokine has been shown to stimulate cell proliferation, promote metastasis, and induce angiogenesis. It also has the ability to indirectly attract the CXCR4-expressing cancer cells to other distant organs which triggers tumour metastasis as shown in Figure 5 (Smith 2011). That being said, zerumbone has been shown to inhibit the effects of CXCL12, as well as modulate the CXCR4 expression in breast cancer cells. In addition, the mRNA expression of CXCR4 is downregulated by zerumbone (Haque et al. 2017; Sung et al. 2008). Through this mechanism, zerumbone indirectly reduces the invasion of breast cancer cells induced by CXCL12, thus acting as a preventive measure against tumour invasion.

Zerumbone effects on Notch signalling

Notch signalling pathway plays a fundamental role in cellular development, especially in determining the fate of the cells in human tissues by influencing either cell proliferation or apoptosis (Lobry et al. 2014). It is found that the hyperactivation of this pathway is associated with cancer progression. For instance, activated Notch1 and Notch3 are overexpressed in transgenic mice which led to mammary tumour formation (Sehrawat, Sakao & Singh 2014). Similarly, aberrant Notch signalling was seen in human breast cancer, suggesting its role in inducing cell proliferation, differentiation and apoptosis (Edwards & Brennan 2021). In 2007, a study described the oncogenic nature of Notch in various cancers, whereby Notch2 downregulation is frequently associated with higher grades of breast cancer (O'Neill et al. 2007). A previous study showed that zerumbone significantly downregulated both cleaved Notch1 and Notch4, which increased cellular apoptosis and inhibited cellular migration (Khera & Gupta 2020; Sehrawat, Sakao & Singh 2014). On the other hand, the same study reported that Notch2 protein level was significantly diminished upon treatment with zerumbone in breast cancer cell lines, suggesting that zerumbone leads to the downregulation of Notch2 mRNA expression. Overall, these findings suggest the inhibitory effect of zerumbone in breast cancer invasion and metastasis via downregulation of Notch 1, Notch2 and Notch4. Zerumbone treatment on breast cancer cells has led to Notch proteolytic cleavage events, ultimately promoting cancer cell apoptosis (O'Neill et al. 2007).

Zerumbone modulates Ras-related C3 botulinum toxin substrate1 (Rac1) expression

Rac1 facilitates several cellular processes such as tumour development, invasion and tumour migration (El Fagie et al. 2021). Previous study speculates the role of Rac1 in the recruitment of neutrophils and inflammation in human tissues, suggesting that tumour invasiveness was markedly affected by impairment of Rac expression (Wertheimer et al. 2012). Growing evidence explains the relationship between P21-activated Kinase 1 (PAK1) pathway and the progression of breast cancer. There were approximately 50% of human breast tumours possess a hyperactivation of PAK1 which indirectly overexpress Rac1 and its active variant, Rac1b, resulting in tumour metastasis (Shrestha et al. 2012; Wertheimer et al. 2012). Hence, two articles clarified that zerumbone significantly reduces the metastatic ability of the malignant cells from invading to distant sites via Rac1 downregulation. The activity of Rac1 protein in breast cancer cells was suppressed upon treatment with zerumbone, which further inhibit tumour migration and induce cell apoptosis (El Fagie et al. 2021; Wertheimer et al. 2012). Consistent with this outcome, another study has shown the inhibitory effect of zerumbone in esophageal squamous cell carcinoma cells (Wang et al. 2019). Nevertheless, the underlying mechanism of zerumbone on Rac1 expression needs further exploration due to the fact that it potentially exhibits anti-cancer properties which may be helpful in treating breast cancer.

Zerumbone impairs BTB and CNC homology 1 (BACH1) level in breast cancer

In 2019, a clinical study has proposed the role of BACH1, a haem-binding transcription factor in the regulation of mitochondrial metabolism to inhibit cancer metastasis. A depletion of BACH1, which normally are overexpressed in cancer, has significantly suppressed the mitochondrial metabolism in breast cancer cells (Padilla & Lee 2021). Particularly, BACH1 inhibit an enzyme called pyruvate dehydrogenase, which functioned to convert pyruvate to acetyl-CoA in glycolysis. Thus, downregulation of BACH1 will reduce the rate of aerobic glycolysis through enzyme inhibition and suppress breast cancer cells metastatic activity. An experiment shows that BACH1 transfection has inhibited the anti-proliferative effects possessed by zerumbone (Zhao et al. 2020). BACH1 was downregulated in zerumbone-treated cells, resulting in the reduction of cell proliferation, migration and invasion. In line with this, the level of BACH1 expression

generally reduced upon zerumbone treatment, but this reduction was reversed when an miR-708 inhibitor was introduced (Zhao et al. 2020). These findings indicate that zerumbone has affected breast cancer by specifically acting on BACH1 and miR-708.

Zerumbone induces BAX- and BAK-mediated apoptosis

The mammary gland was reported to express a variety of B-cell lymphoma 2 (BCL-2) relatives including BCL-X, BAX, BAK and BCL-W. Depending on the oestrogen receptor, BCL-2, which is markedly expressed in solid tumours such as breast cancer, exhibits an anti-apoptotic property which is essential for breast cancer prognosis (Eom et al. 2016). Evidence on the role of zerumbone in mediating BCL-2-induced apoptotic pathway in breast cancer is limited. Hence, in 2012, a finding apparently showed that zerumbone treatment on breast cancer cell lines had reduced both BAK and BAX expression (Sehrawat et al. 2012). Additionally, little information was found, showing the elevated pro-apoptotic effect of BAX and reduced anti-apoptotic effect of BCL-2 protein in liver cancer cells upon treatment with zerumbone (Sakinah, Tri Handayani & Hawariah 2007). Hence, previous findings indicate that apoptosis-induced mechanisms by zerumbone involve the BCL-2-mediated pathway. More exploration may be required to understand the underlying mechanisms of this protein in breast cancer progression.

Proteins involved in breast tumor metastasis upon treatment with zerumbone

Many studies have gathered substantial evidence indicating that zerumbone has the potential to reduce migration, invasion and metastasis of breast cancer cells through various mechanisms as shown in Figure 6. A summary of mechanisms by which zerumbone acts on breast cancer cell metastasis is provided in Table 2.

TABLE 1. Several pharmacological properties of zerumbone

Model	Finding(s)	Pharmacological property	References
Rat normal liver epithelial cell line (RL34 cells)	• ↑ GSH level • Inhibition of lipid peroxidation	Antioxidative	(Nakamura et al. 2004)
RAW 264.7 murine macrophages	• Suppression of iNOS/COX-2 gene expression via IκB degradation	Anti-inflammatory	(Murakami et al. 2003)
Adult male ICR mice	• Inhibition of carrageenan-induced paw oedema	Anti-inflammatory	(Sulaiman et al. 2010)
HCl-induced gastric lesions in Swiss albino mice	• Potent cytoprotective effect against HCl and indomethacin-induced gastric ulceration • Moderate cytoprotective effect against ethanol-induced gastric lesions	Anti-ulcer	(Al-Amin, Sultana & Hossain 2012)
Methicillin-resistant S. aureus (NCTC 13277)	• ↑ membrane permeability • Cell membrane disruption • MIC value of 125 µg/ml against *Methicillin-resistant S. aureus*	Anti-microbial	(Fadhel et al. 2022)

GSH=glutathione; MDA=malondialdehyde; TBARS=thiobarbituric acid reactive substance; iNOS=inducible nitric oxide synthase; COX-2=cyclooxygenase-2; IκB=inhibitor kappaB; HCl=hydrochloric acid; PGE2=prostaglandin E2; NP-SH=non-protein sulfhydryl gastric; Hsp70=heat shock protein 70; AST=aspartate transaminase; ALT=alanine aminotransferase

TABLE 2. Proteins involved in cancer cell metastasis after treatment with zerumbone

References	Proteins	Mechanism of action
(Sung et al. 2008)	CXCR4 and CXCL12	↓ CXCR4, CXCL12
(Sehrawat, Sakao & Singh 2014)	Notch1, Notch2 and Notch4	↓ Notch1, Notch4, Notch2
(Jeon et al. 2016)	IL-β, MMP-3	↓ IL-β, MMP-3
(Kim et al. 2015)	FN, MMP-2, MMP-9	↓ FN, MMP-2, MMP-9
(Kim et al. 2014)	CD44	↓ CD44
(Shyanti et al. 2017)	CD1d	↓ CD1d
(Wang et al. 2019)	Rac1	↓ Rac1
(Zhao et al. 2020)	BACH1	↓ BACH1 ↑ miR-708 regulation
(Sehrawat et al. 2012)	BCL-2	↓ BCL-2

CXCR4= CXC chemokine receptor 4; CXCL12= CXC chemokine ligand 12; IL-β= interleukin-β; MMP= matrix metalloproteinases; FN= fibronectin; CD44= cluster of differentiation 44; CD1d= cluster of differentiation 1d; Rac1= Ras-related C3 botulinum toxin substrate1; BACH1= BTB and CNC homology1; miR= microRNA; BCL-2= B-cell lymphoma 2

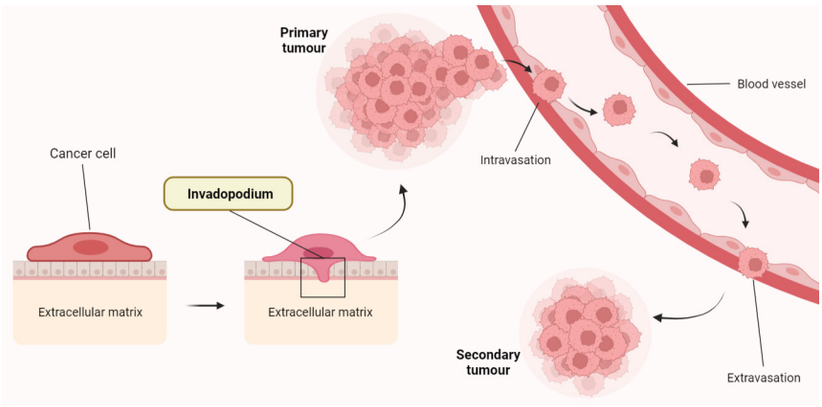

FIGURE 1. Invadopodia formation contributes to cancer cells metastasis (modified and adapted from Liu, Semenza & Zhang 2015)

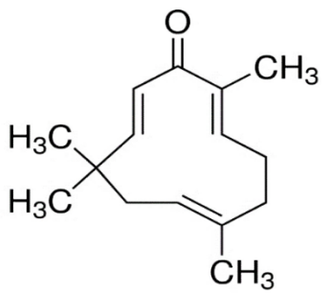

FIGURE 2. Chemical structure of zerumbone compound (adapted from Girisa et al. 2019)

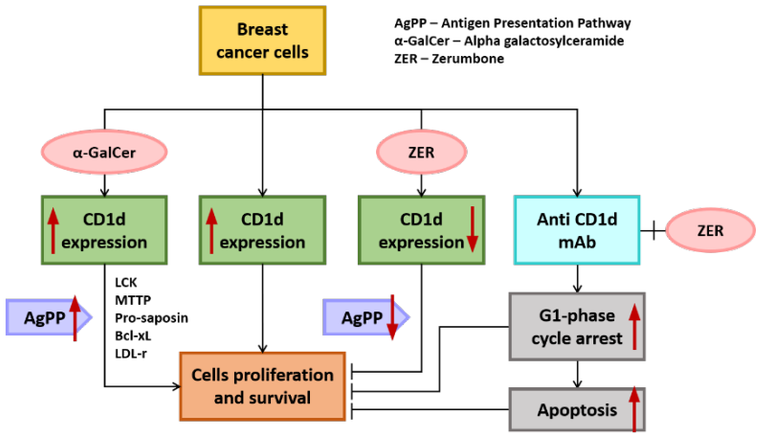

FIGURE 3. Decrease of CD1d expression in breast cancer upon zerumbone treatment (adapted from Shyanti et al. 2017)

α-GalCer= Alpha galactosylceramide; ZER= zerumbone; CD1d= cluster of differentiation 1; AgPP= Antigen Presentation Pathway; LCK= lymphocyte-specific protein tyrosine kinase; MTTP= microsomal triglyceride transfer protein; BCL-xL= B cell lymphoma-extra-large; LDL-r= low-density lipoprotein receptor; mAb= monoclonal antibody

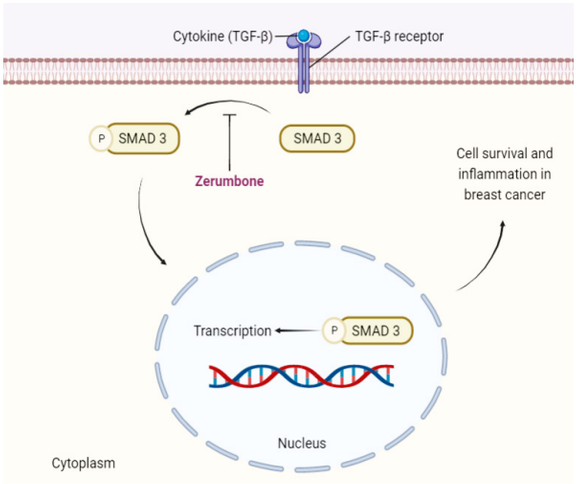

FIGURE 4. Zerumbone attenuates the expression of TGF-β in breast cancer (modified and adapted from Khoshakhlagh et al. 2019)

TGF-β=transforming growth factor beta; SMAD3=SMAD family member 3

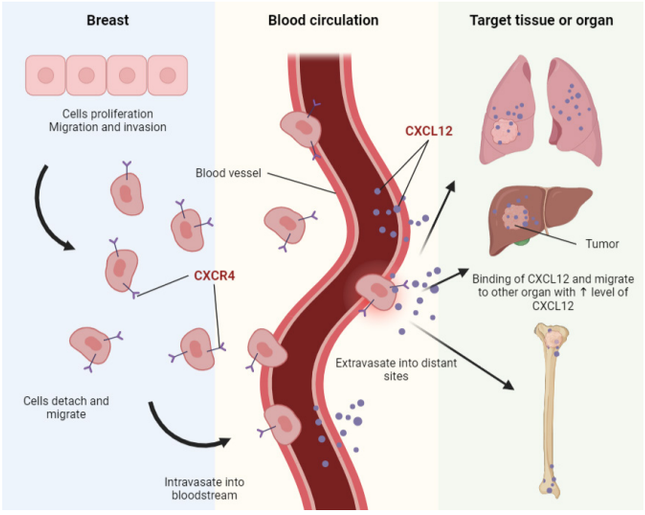

FIGURE 5. CXCR4/CXCL12-mediated metastatic pathway in breast cancer progression (modified and adapted from Smith 2011)

CXCR4=CXC chemokine receptor 4; CXCL12=CXC chemokine ligand 12

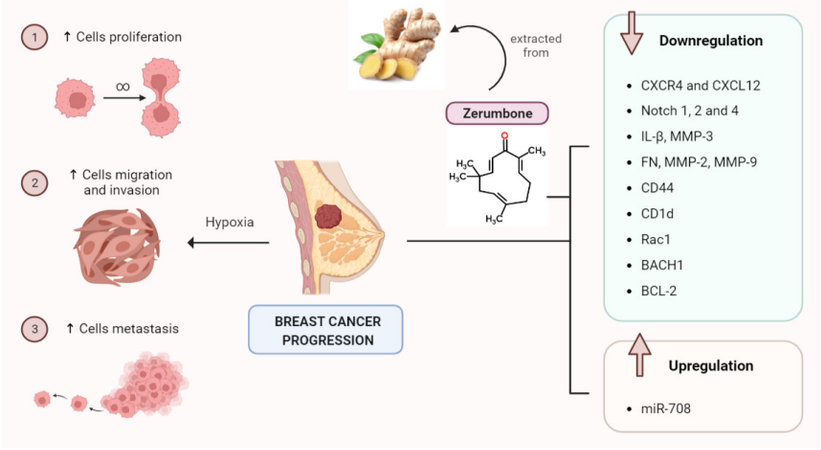

FIGURE 6. Summary of zerumbone effects in decreasing cells migration, invasion and metastasis in breast cancer by acting upon invasion-related proteins

CXCR4=CXC chemokine receptor 4; CXCL12=CXC chemokine ligand 12; IL-1β=interleukin 1 beta; MMP-3=matrix metalloproteinase 3; MMP-2=matrix metalloproteinase 2; MMP-9=matrix metalloproteinase 9; FN=fibronectin; CD44=cluster of differentiation 44; CD1d=cluster of differentiation 1d; Rac1=Ras-related C3 botulinum toxin substrate 1; BACH1=BTB and CNC homology 1; BCL-2=B-cell lymphoma 2; miR-708=microRNA-708

Conclusion

In conclusion, there has been growing interest in the potential therapeutic of Zerumbone against breast cancer. Zerumbone acts upon multiple signalling pathways such as ERK/AKT pathway, SMAD pathway, STAT pathway and NF-κB pathway. It reduces the expression levels of CD1d, TGF-β, CD44 and IL-1β, Rac1, BACH1, Notch signalling, CXCR4 and BCL-2 which mainly contribute to the invasion and metastasis of cells. Intriguingly, the formation of invadopodia is one of the mechanisms directly involved in cancer cell invasion and metastasis owing to its ECM degradation capabilities. The expression of HIF-1α, a molecular factor that regulates hypoxia, is significantly higher in breast cancer tissues as it mediates angiogenesis and metabolic reprogramming, both of which are involved in the progression of malignancies. Nevertheless, the effects of zerumbone in regard to invadopodia formation and HIF-1α expression remain poorly understood. Therefore, more research must be conducted in these areas to further elucidate its mechanisms of action. With that, a clearer therapeutic potential of zerumbone may be established, possibly rendering this phytochemical a potential treatment against breast cancer in the future.

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