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Anticancer Potential of Chalcone and its Derivatives Against Triple Negative Breast Cancer

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ABSTRACT: Clinical, physical, and molecular aspects of breast cancer vary widely. The expression of the estrogen receptor (ER), the progesterone receptor (PR), the human epidermal receptor 2 (HER2), and Ki-67 is used to classify breast cancer into luminal A, luminal B, triple-negative, and HER2 overexpression subtypes. Triple-negative breast cancer (TNBC) is one of the most challenging cancers to treat because of its complicated pathophysiology and uncertain prognosis. The absence of the primary targets of ER, PR, and HER2 expression status required by most breast cancer drugs complicates the treatment of TNBC. Based on historical data, chalcone chemicals and their derivatives are solid prospects for chemotherapeutic development. Chalcone (1,3-diaryl-2-propen-1-one) and its derivatives have shown anticancer activity in various developmental processes and signaling pathways in TNBC, including apoptotic induction, cell cycle arrest, suppression of epithelial-mesenchymal transition (EMT), blockage of beta-catenin, and prevention of angiogenic and metastatic development. Chalcone also has good selectivity towards normal cells. Animal studies have also shown a reduction in tumor size. Therefore, chalcone compounds and their derivatives have good potential as anticancer.

Abbreviations: Bax: Bcl-2-associated protein; Bcl-2: B-cell lymphomna 2; CAM: chick chorioallantoic membrane; Cdc25: cell division cycle 25; CDK: cyclin-dependent kinase; DNA: Deoxyribonucleic acid; EMT: epithelial-mesenchymal transition; ER: estrogen receptor; FAK: focal adhesion kinase; GSK3b: glycogen synthase kinase 3 beta; HER2: human epidermal receptor 2; IC_{50}: inhibitory concentration 50%; LPR: low-density lipoprotein-related receptor; MMP: matrix metalloproteinase; OS: overall survival; PARP: Poly (ADP-ribose) polymerase; pCR: pathological complete response; PR: progesterone receptor; SCID: severe combined immunodeficient; Tcf/Lef: T-cell factor/lymphoid enhancer factor; TNBC: triple-negative breast cancer; Wnt: Wingless–Int; ZNRF3: zinc and ring finger 3.

1. Introduction

According to the Global Cancer Observatory (GLOBOCAN), in 2020, breast carcinoma was accounted for 24.5 percent incidence and the leading cause of mortality from cancer in women. According to the Global Cancer Observatory

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(GLOBOCAN) in 2020 [1]. About 2.3 million women are diagnosed with breast cancer which causes 685,000 deaths globally [2]. In Indonesia, breast cancer was ranked first in the number of new cases in 2020, which amounted to 65,858. In the same year, the number of deaths from breast cancer in Indonesia was 22,430 [3].

In terms of clinical, pathological, and molecular characteristics, breast cancer is quite diverse. The immunohistochemical examination is the standard in determining breast cancer subtypes that can be used to determine therapy and prognosis. It is common to classify breast cancer into luminal A, luminal B, triple-negative, and HER overexpression based on ER, PR, HER2, and Ki-67 expression levels [4].

Among all types of breast carcinoma, type of cancer, triple-negative breast cancer (TNBC) accounts for about 10-15% [5]. TNBC cancer cells do not express estrogen (ER), progesterone (PR), and HER2 receptors [6]. TNBC has a high risk of recurrence and death [7]. TNBC usually shows a zone of central necrosis and irregular borders due to the infiltration of the surrounding tissue. Triple-negative tumors often show fibrous cellular proliferation. Blood vessels of various sizes, including vessels with thick walls, are common in TNBC [8].

Due to its high early recurrence rate and tendency to spread to distant organs, TNBC has a much worse prognosis than other breast cancer types [9]. Studies on breast cancer mortality based on ER/PR/HER2 subtypes at each stage show that TNBC has the worst survival rate [10]. Patients with TNBC are more likely to have a recurrence 1 to 4 years after diagnosis than other types of cancer [11].

The hallmark of TNBC is the absence of ER, PR, and HER2, thus rendering it to some of the most effective therapies, such as HER2-targeted therapy and hormone therapy. The lack of known specific therapeutic targets limits TNBC therapy. The primary treatment for TNBC consists of standard cytotoxic chemotherapy [12].

Chemotherapy used in the treatment of TNBC are anthracycline, taxane, alkylator, and others, but the results are not satisfactory. In TNBC treated with neoadjuvant chemotherapy, 20-30% of patients achieve a pathological complete response (pCR) and have an excellent overall survival (OS) rate. In contrast, patients with TNBC who did not achieve pCR had a lower OS and higher recurrence rate.(13) In one study, only 11.4 percent of individuals with TNBC achieved a 5-year progression-free survival rate [14]. Generally, TNBC is sensitive to chemotherapy, especially when given preoperatively, but the results are poor in most patients. The poor result shows that therapy is less than optimal in inhibiting cancer development [9].

Because most breast cancer treatments need the expressions of ER, PR, and HER2 targets to be present, and resistance to chemotherapeutic agents complicates the treatment of TNBC. Various trials were conducted to test the efficacy of multiple approaches to TNBC therapy. Chalcone chemicals and their derivatives may be used to combat cancer, based on historical data for developing anticancer drugs [15].

2. Chalcone

Chalcone (1,3-diaryl-2-propen-1-ones) is a precursor in the biosynthesis of flavonoids/ isoflavonoids, which are widely found in vegetables, and are easily obtained by humans through rich fruits and vegetables. With three carbon chains connecting two aromatic rings, chalcosides are open-chain flavonoids known as Chalcones (Fig. 1). There are two types of chalcones: cis and trans isomeric types. The thermodynamically favoured form is trans. Presumably, the unsaturated ketone constituent, which has a defective structure, is accountable for the biological properties that have been identified [18].

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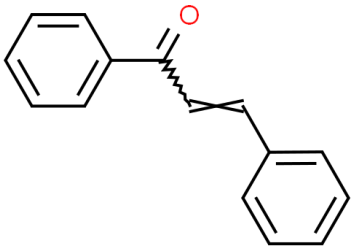

Figure 1. Basic Structure of Chalcone [18]

Natural or synthetic chalcone analogues have been shown to inhibit the growth and spread of TNBC and other malignancies through diverse signaling pathways and cancer formation processes [17].

2.1. Chalcone induces apoptosis in cancer cells

Chalcone can induce apoptosis via intrinsic (mitochondria) and extrinsic pathways. An imbalance between anti-apoptotic (e.g., Bcl-2) and pro-apoptotic (e.g., Bax) proteins will activate Bax, which will increase mitochondrial permeability. Apoptosis is initiated when pro-apoptotic proteins, such as cytochrome c, are discharged from the inner membrane of the mitochondria as a result of mitochondrial damage, activating caspase-3. DNA fragmentation was observed in cells undergoing apoptosis, thus also used as a biomarker of apoptosis. Cardamonin, a natural chalcone compound, exhibited apoptosis in BT-549 and MDA-MB-231 cells characterized by mitochondrial membrane damage and DNA fragment formation [19].

Novel chalcone analogues, comprising alicyclic amines or nitrogen mustard substituents, were used to treat MDA-MB-231, MDA-MB-46, and BT-549 cell lines. The results showed blebbing of the membrane, apoptotic bodies, and cell death. Phosphatidylserine translocation from the inner plasma membrane to the outside of the cell, indicating an early stage of apoptosis, was also observed. There was a significant increase in apoptosis by up-regulation of the pro-apoptotic Bcl-2-associated X protein (BAX) and down-regulation of the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2). The nitrogen group in the compound may have an effect because it can act as a DNA alkylating agent and interfere with cell cycle control [20].

(E)-3-[3-(4-methoxyphenyl)quinolin-2-yl]-1 (3,4,5-trimethoxyphenyl)prop-2-en-1 -one showed a potent activity towards the proliferation of MDA-MB-231 cells, with an IC50 value of 0.75. It induces cell apoptosis by increasing apoptotic Bax protein and decreasing anti-apoptotic protein Bcl-2. The chemical promotes cell death by inducing Poly (ADP-ribose) polymerase (PARP) breakage and caspase-3 and-8 activation [21].

This route is known as the extrinsic or death receptor pathway because it involves initiator caspase-8 and caspase-10. From *Cyathostemma argenteum*, a dihydrochalcone was obtained that has a hydroxy-2′,4′-dimethoxy-5′,2″ hydroxy-benzyl groups. Caspase-3 and caspase-8 activity in MDA-MB-231 cells enhanced considerably after 24 hours of treatment with compound 1, particularly at the IC_{50} (232.7 µM) dose [22].

2.2. Chalcone causes cell cycle arrest in breast cancer cells

The cell cycle in healthy cells is strictly regulated, unlike tumor cells, which are unresponsive to regulatory mechanisms and divide uncontrollably. Several cell cycle regulatory proteins are cyclins and cyclin-dependent protein kinases (CDKs), which are activated by Cdc25 phosphatase [23]. Inhibition of cyclin B1/CDK1 activation by Cdc25 will result in cell cycle arrest in the G2/M phase. Cells treated with 3-phenyl-quinolinyl chalcones and SL4, a chalcone derivative, showed a decrease in Cdc25, CDK1, and cyclin B1, which regulates cell cycle arrest in the G2/M phase [21, 24]. CDK inhibitor p21, which SL4 increases, can potentially interrupt the cell cycle in the G2/M phase [24, 25].

Microtubules play an important role in motility, formation of the mitotic apparatus and cell division, angiogenesis, metastasis, cell structure, and transport within cells. Drugs that act to inhibit

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tubulin polymerization or inhibit its depolymerization will cause cell cycle termination in the G2/M phase and followed by apoptosis [23]. Inhibition of tubulin polymerization in MDA-MB-231 cells was observed after treatment with 3-phenyl quinolinyl chalcone derivatives [21]. TUB091, a newly synthesized chalcone, binds to tubulin's colchicine site and distorts microtubules. When the spindle depolymerizes, the DNA does not line up at the equator at metaphase as it should. The number of metaphase cells with aberrant mitotic spindles was observed following treatment with chalcone [26].

PARP is an enzyme that catalyses the transfer of ADP-ribose to various target proteins, such as proteins involved in DNA repair. Cells with DNA strand breaks are prevented from starting mitosis by PARP's involvement in cell cycle checkpoint G2. A decrease in PARP will lead to an increase in damaged cells and a halt in the cell cycle [27]. PARP cleavage was seen after treatment with cardamonin and 3-phenyl quinolinyl chalcone [19, 21].

2.3. Chalcone inhibits Epithelial to Mesenchymal Transition (EMT) in triple negative breast cancer cells

Tumor cell infiltration and metastasis are characterized by the epithelial to mesenchymal transition or EMT. Epithelial cells lose cell-cell adhesion and tight junctions during this phase. In contrast, the cells become characterized by mesenchymal fibroblasts that could increase tumorigenesis, invasion, and metastasis [15]. Cadherin switch from E-cadherin, an epithelial marker, to N-cadherin, a mesenchymal marker expressed on mesenchymal, fibroblasts, nerve, and cancer cells, is often used to monitor EMT during cancer progression [28].

Cardamonin and (E)-3-(4-(Bis(2-chloroethyl) amino) phenyl)-1-(3-methoxyphenyl) prop-2-en-1-one) may promote cell adhesion and inhibit cancer invasion by up-regulating E-cadherin and causing decreased expression protein Focal adhesion kinase (FAK) [15,19]. FAK is a cytoplasmic protein tyrosine kinase associated with integrins and growth factor receptors that play a role in the adhesion, migration, and invasion of cancer cells [29]. Downregulation of mesenchymal markers, such as N-cadherin, vimentin, slug, and snail, also occurs. Slug and snail are transcription factors that inhibit E-cadherin expression [19].

2.4. Chalcone inhibits β-catenin in triple negative breast cancer cells

Beta-catenin (β-catenin) is a protein that plays a role in cell development under physiological conditions. β-Catenin is a critical transcription factor in the Wingless–Int (Wnt) signaling pathway. Normal cells may become malignant when β-catenin is overexpressed, which has been seen in many malignancies.(30) WNT protein binds to frizzled receptors (G protein receptor family), and LPR co-receptors suppress glycogen synthase kinase-3β activity. (GSK-3β). ZNRF3 promotes WNT receptor degradation and functions as a tumor suppressor. The process suppressed phosphorylation of downstream signaling molecules and accumulation of β-catenin in the cytoplasm. Nucleoplasmic translocation and transcription are initiated by β-catenin's interaction with the Tcf/Lef transcription complex. In breast cancer, WNT/β-catenin signaling drives cancer stem cell self-renewal and migration, promoting tumor development and metastasis [31].

Cardamonin inhibits the phosphorylation of GSK3b by Akt, thereby restoring the activity of glycogen synthase kinase-3b (GSK3b), which is required for the formation of a destruction complex so that the proteasome degrades β-catenin, thereby inhibiting proliferation and EMT [19].

2.5. Chalcone increases angiogenesis and metastasis in triple negative breast cancer cells

Increased mobility of cancer cells is related to their metastatic potential. Invasion and metastasis of cancer are linked to matrix metalloproteinase (MMP) enzymes. A variety of cancer cells release MMP-9, which aids tumor dissemination by

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cleaving extracellular matrix molecules, enabling metastatic cells to spread farther. BDP, a novel chalcone-based molecule, significantly inhibited MMP-9 activity, inhibiting the migratory and invasive ability of MDA-MB-231 cells [32].

Chick chorioallantoic membrane (CAM) is an extra-embryonic membrane that contains many blood and lymph vessels. CAM was used to study the effect of drugs on angiogenesis *in vivo* [33]. The phenyl ring of (E)-3-(4-(Bis(2-chloroethyl) amino) Propyl-3-methoxyphenyl 2-en-1-one) was shown to have a substantial impact on the CAM compared to the control group.(20) In the CAM experiment, TUB091 prevented endothelial cell migration and angiogenesis. This chemical also degrades blood vessels still in the early stages of development [26].

Spontaneous metastases were observed after treatment and removal of the primary tumor. In the control group, some tumors reappeared, but in the chalcone group, the animal subjects remained tumor-free. The chalcone group had no metastases four weeks following tumor removal, while 87 percent of the control group's individuals had lung and/or lymph node metastases during the same period. After initial tumor excision, TUB091 is a helpful treatment for preventing spontaneous metastasis [26].

3. Potential development of chalcone as an anticancer

The search for anticancer agents from synthetic and natural products has been widely carried out. Compounds isolated from plants such as vinca alkaloids (Vincristine, Vinblastine) from *Catharanthus roseus*, Paclitaxel from *Taxus brevifolia*, Camptothechin from *Camptotheca acuminata*, Podophyllotoxin from *Podophyllum peltatum*, is known to have cytostatic and cytotoxic activity [34]. These compounds have been used as chemotherapeutic agents against various cancers, including breast cancer. Chalcone is a naturally occurring compound found in various plant species. Studies have shown that chalcone has antimicrobial, antibiotic, antiparasitic, antimalarial, anti-inflammatory, and anticancer activities. Metochalcone and Sofalcone have been used as choleretics and mucoprotectant, respectively [35].

Chalcone also has good selectivity. Chalcone derivatives were tested on normal cells to determine the selectivity of proliferation inhibition. Studies have shown that chalcone derivatives selectively kill TNBC cells without affecting normal breast epithelial cells (MCF-10A) [19]. Studies by Solomon and Lee showed a significant increase in antiproliferative activity by their synthesized chalcone derivatives against MDA-MB231 and MDA-MB468 with selectivity. Its activity is 3 to 7 times higher in cancer cells than in normal cells [36]

In vivo studies have also been carried out on TNBC-induced mice to test the efficacy of chalcone. Tests were carried out to see if chalcone can inhibit tumor development in Balb/c mice by creating a breast cancer model (4T1). Administration of Cardamonin at the dose of 5 mg/kg significantly suppressed tumor volume compared to the control group [19]. *In vivo* study on SCID mice induced with MDA-MB-231 cells and then given DJ52, a novel isochalcone, at a dose of 50 mg/kg, caused a significant decrease in tumor volume up to 50% compared to control ($p < 0.05$) [37]. This indicates that chalcone can inhibit tumor growth *in vivo*.

Taken together, chalcone has promising anticancer potentials. Among the things that are impacted are apoptosis, cell cycle arrest, the epithelial-mesenchymal transition (EMT), blocking β-catenin, angiogenesis, and metastasis, as illustrated in Figure 2.

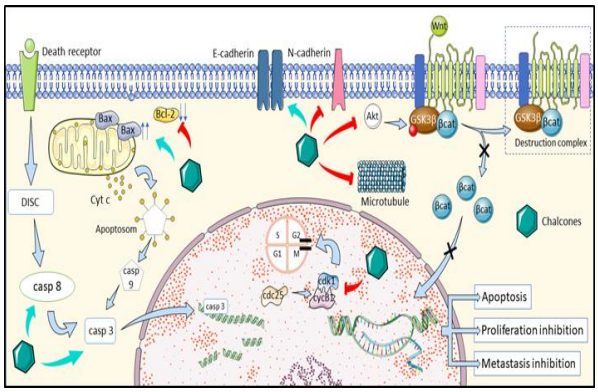

Figure 2. Multiple mechanisms of Anti-Cancer Activity of Chalcones

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4. Conclusion

Chalcone shows promising anti-cancer properties. In TNBC, natural chalcone chemicals and their derivatives exhibit anticancer efficacy in a variety of signaling pathways and processes that promote cancer growth. Apoptosis, cell cycle arrest, EMT, β-catenin inhibition, angiogenesis, and metastasis are among the factors impacted. A computer-assisted drug design approach can be used in designing and developing chalcone as an effective anticancer drug candidate. TNBC is a complex illness with a wide range of clinical, pathological, and molecular symptoms. Thus, careful study of the off-target impact, specificity, toxicity, and several other factors is necessary. Appropriate synthesis techniques also need to be developed. It is also necessary to conduct further research and clinical trials to evaluate chalcones' efficacy, interactions, and safety profile.

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