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Review

Multiple molecular targets in breast cancer therapy by betulinic acid

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ABSTRACT

Breast cancer is the second most common type of cancer in the world, and is by far the most prevalent form of cancer in women. However, the efficacy of current treatments for breast cancer is limited. In addition to the high risk of recurrence, some of these have side effects that significantly reduce the quality of life. Therefore, new avenues of treatment for breast cancer are needed. Betulinic acid (BA), a pipeline anticancer drug, exerts anti-proliferative effects on breast cancer cells is mainly through inhibition of cyclin and topoisomerase expression, leading to cell cycle arrest. It induces apoptosis through mitochondrial pathway and anti-angiogenesis effect by inhibiting the expression of transcription factor nuclear factor kappa B (NF-κB), specificity protein (Sp) transcription factors, and vascular endothelial growth factor (VEGF) signaling. In addition, it exerts anti-metastatic effect by inhibiting the expression of matrix metalloproteases. The specific targets of BA in breast cancer are reported to be the estrogen receptor and various multidrug resistance proteins. Synergistically interactions of BA with other chemotherapeutics are also described in the literature. In this review, we describe the detailed published mechanisms of action of BA, a pentacyclic triterpene with a lupine skeleton, on multiple molecular targets to treat breast cancer. We hope that this review will provide basic information in support of future studies of effects of BA on breast cancer cells.

Contents

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1. Introduction

Breast cancer is the second most common type of cancer in the world, and is by far the most prevalent form of cancer among women. The morbidity of it was so high in 2012 that approximately 1.67 million women were diagnosed with breast cancer [1]. Among all types of cancer, breast cancer has the fifth highest mortality rate. It is the most frequent cancer-related cause death among women in developing countries, and the second most frequent among women in developed countries [1]. Multiple factors are involved in breast cancer, including genes, hormones, nutrition, and obesity, although the mechanisms by which these factors influence risk on breast cancer are not completely clear [2]. Thus, changing bad dietary habits, could reduce breast cancer risk. The current standard of breast cancer therapy is surgery, in conjunction with radiotherapy, chemotherapy, hormone therapy, and/or immune stimulant treatment [3]. The efficacy of current treatments for breast cancer is limited. In addition to significant side effects of therapy, the risk of recurrence is high [4]. Therefore, finding more effective treatments with fewer, less-severe side effects are necessary.

Betulinic acid (3β, hydroxyl-lup-20(29)-en-28-oic acid, BA) (Fig. 1) is a pentacyclic triterpene with a lupine structure [5]. It can be found in plant sources including white birch bark [6], acuminatissima leaves, and wild jujube seeds. Likewise, it can be obtained from betulin (lup-20(29)-ene-3β, 28 diol) (Fig. 1), a triterpene isolated from the same species of BA [7]. There are three reactive positions on the structure of betulin, and new derivatives could be produced via chemical modifications of these structures. The reactive positions are a secondary hydroxyl group at C-3, a primary hydroxyl group at C-28, and a C-C double bond at C-20 [5]. When the C-28 hydroxyl group is oxidized to a carboxyl group, betulin can be rapidly converted to BA [7]. This method is currently used industrially. Studies have demonstrated that BA has many different biologic properties: anti-inflammatory, anti-oxidative, anti-malarial [8], anti-HIV [9], anti-angiogenic, anti-proliferative, and cytotoxic towards various cancers cells [10], including breast cancer [3], lung cancer [11], prostate cancer [12], skin cancer [13], and gastric cancer [14]. Experiments reveal that BA can inhibit viability and migration of breast cancer cells, arrest cell cycles at G1 phases, induce mitochondrial pathway of cell apoptosis, and inhibit angiogenesis [3,4]. The direct targets of BA in breast cancer cells include topoisomerase [15], transcription factor nuclear factor kappa B (NF-κ B) [16], specificity protein transcription factors (Sp) [17], the ZBTB family [18], vascular endothelial growth factor (VEGF) and its receptor (VEGFR) [19], estrogen receptors (ER) [20], and multidrug resistance proteins (MDRP) [21].

Many drugs have carcinogenic potential in human cells [22]. However, experiments have demonstrated that BA selectively acts on cancer cells, not normal cells [23,24]. It means that BA is a very promising pipeline anticancer drug with pharmacological safety [25].

In this review, we focus on the promising and pharmacologically safe drug, BA and its efficacy towards breast cancer treatment through various targets. We will also discuss new studies that help to clarify BA's mechanisms of action.

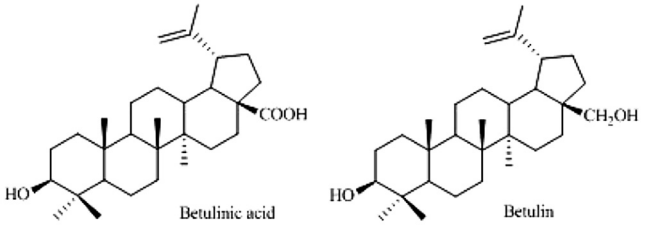

Fig. 1. The chemical structure of betulinic acid and betulin.

2. Anti-proliferative effect of BA by inhibition of cyclin activity

2.1. Inhibition of breast cancer cell proliferation by BA

BA has potent anticancer activity in many kinds of tumor cells, including colon cancer, prostate cancer, skin cancer, liver cancer, lung cancer and breast cancer cell lines [26,27] (Table 1). It suppresses growth, proliferation, and viability of breast cancer cells [28,29], arrests cells at G1 phase and induces apoptosis, without any cytotoxic effect on normal cells [30].

BA can suppress the proliferation of many kinds of breast cancer cell lines, including MCF-7, MDA-MB-231, MDA-MB-453, BT474 and T47D [31–34]. At 10 μM, BA can inhibit growth of MCF-7 cells [35].

2.2. Mechanism of BA-induced cell cycle arrest in breast cancer cells

BA can induce cell cycle arrest in breast cancer cell lines [36,48]. For example, MCF-7 cells arrest in G1 when treated with BA [4]. One mechanism might be cell cycle arrest triggered by decreased mitochondrial ATP production to arrest cell cycle [49,50]. Alternatively, BA increases expression of p53 and p21, which are involved in cell cycle regulation, and can arrest MCF-7 in G1 phase [51]. MDA-MB-231 and T47D cell lines lacking p53 arrest in G1 phase under BA treatment through activation of p21 [32]. BA decreases expression of Bcl-2 family anti-apoptotic proteins (Bcl-2, Bcl-XL), and increases expression of pro-apoptotic proteins Bax, Bak and Mcl-1, increasing the ratio of Bax/Bcl-2, these changes in gene expression also promote cell cycle arrest at G1 [51]. Cyclin D1, an important protein for cell cycle progression in breast cancer, is inhibited by BA through suppression of NF-κB [52], or by down-regulated expression in T47D cells [43,53,54].

2.3. Anticancer effects of BA in vivo

BA exerts its effects on breast cancer not only *in vitro*, but also *in vivo* [4,55], especially in the condition of hypoxia [56]. It suppresses tumor growth and increases survival time in an animal cancer model [23]. BA reduces the size and weight of tumors, possibly through inhibition of angiogenesis in mouse models of human breast cancer [31] and other cancers including human colon carcinoma [44,57] and human liver cancer [58]. In addition, BA reduces immune tolerance caused by apoptosis in cancers [59]. Betulin, the precursor of BA, produces similar results in *in vivo* models [60]. BA was to found to exhibit significant efficacy in melanoma in phase I and II clinical trial [61,62].

These findings indicate BA has efficacy *in vitro* and *in vivo* in treating breast cancer without any cytotoxic effects on normal cells and tissues.

3. Inhibition of topoisomerase activity

DNA topoisomerases plays a vital role in maintaining the integrity of chromosome structure during replication, recombination, and transcription via relaxing DNA supercoiling [63,64]. Eukaryotic have two types of topoisomerases [64]. Topoisomerase I mainly affects replication through forming transient single-stranded breaks in DNA. Topoisomerases II simultaneously breaks both strands in the first step of its enzymatic activity [65,66]. Topoisomerases II is involved in growth, proliferation, and apoptosis of cells.

BA was recently shown to inhibit the function of topoisomerase I and II in cancer cells [37]. BA mediated inhibition of