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TOPIC HIGHLIGHT

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Effects of psoralens as anti-tumoral agents in breast cancer cells

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Abstract

This review examines the biological properties of coumarins, widely distributed at the highest levels in the fruit, followed by the roots, stems and leaves, by considering their beneficial effects in the prevention of some diseases and as anti-cancer agents. These compounds are well known photosensitizing drugs which have been used as pharmaceuticals for a broad number of therapeutic applications requiring cell division inhibitors. Despite this, even in the absence of ultraviolet rays they are active. The current paper mainly focuses on the effects of psoralens on human breast cancer as they are able to influence many aspects of cell behavior, such as cell growth, survival and apoptosis. In addition, analytical and pharmacological data have demonstrated that psoralens antagonize some metabolizing enzymes, affect estrogen receptor stability and counteract cell invasiveness as well as cancer drug resistance. The scientific findings summarized highlight the pleiotropic functions of phytochemical drugs, given that recently their target signals and how these are modified in the cells have been identified. The encouraging results in this field suggest that multiple modulating strategies based on coumarin drugs in combination with canonical chemotherapeutic agents or radiotherapy could be a useful approach to address the treatment of many types of cancer.

Key words: Psoralens; Breast tumor; Bergapten; Growth factors; Estrogens

Core tip: This review examines the biological properties of coumarins by considering their beneficial effects in the prevention of some diseases and as anti-cancer agents. The attention is mainly focused on the effects of psoralens on human breast cancer as they are able to influence many aspects of cell behavior. More recently, it has been reported that these drugs in breast cancer cells are capable of antagonizing some metabolizing enzymes, to affect estrogen receptor stability and to counteract cell invasiveness as well as cancer drug resistance.

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INTRODUCTION

Coumarins are classified as members of the benzopyrone family of compounds, all which consist of a benzene ring joined to a pyrone ring[1]. There are four main coumarin sub-types: the simple coumarins (*e.g.*, coumarin, 7-hydroxycoumarin and 6,7-dihydroxycoumarin); the furanocoumarins (*e.g.*, psoralen, angelicin) that consist of a five-

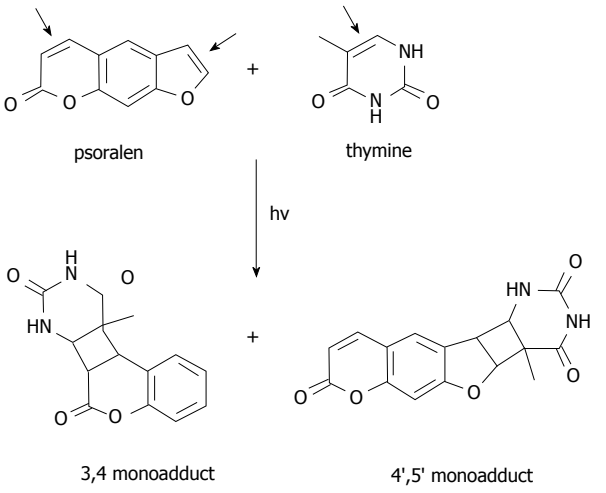

Figure 1 Interaction between psoralen and DNA. Upon absorption of ultraviolet radiation A (UVA) photons, the psoralen excited state can react with a thymine and covalently attach the DNA. The initial photoadduct can absorb a second UVA photon and react with a second thymine on the opposing strand of DNA helix to crosslink the two strands.

membered furan ring attached to the coumarin nucleus, divided into linear or angular types with substituents at one or both of the remaining benzoid positions; the pyranocoumarins that are analogous to the furanocoumarins but contain a six-membered ring (*e.g.*, seselin, xanthyletin); and the coumarins substituted in the pyrone ring, often at 3-C or 4-C positions (*e.g.*, warfarin)[2].

Coumarins comprise a very large class of compounds found throughout the plant kingdom[3-5]. They are present in fruit, roots, stems and leaves, but some essential oils, particularly cinnamon bark, lavender oil and cassia leaf oil, are also a good source. Coumarin members have also been isolated from microorganisms. For example, coumarin group antibiotics, such as novobiocin, coumermycin A1 and clorobiocin, come from various Streptomyces species. These antibiotics are potent inhibitors of DNA gyrase[6,7]. Aflatoxins, isolated from the Aspergillus species, are a group of highly toxic fungal metabolites.

In the 1970s, furanocoumarins attracted scientific attention when they were introduced in clinical practice[8]. Two of the most important and well known furanocoumarins are psoralen and angelicin, which have been demonstrated to influence cell division and differentiation. In addition, anticancer[9,10], immunomodulatory[11], antibacterial[12], antioxidant[13] and neuroprotective functions[14] have also been shown.

Psoralens, extremely toxic to a wide variety of prokaryotic and eukaryotic organisms, are mainly extracted from the ripe fruit of Psoralea corylifolia (Leguminosae), an erect annual herb widely used in Ayurvedic medicine and traditional Chinese medicine. 8-methoxypsoralen (8-MOP) and 5-methoxypsoralen (5-MOP), or bergapten, are the psoralen compounds that occur in nature but several analogues have also been described[15-20].

These molecules cause cell damage by covalent binding to DNA after ultraviolet radiation A (UVA) irradiation. They have a planar tricyclic structure with two photoreactive sites (3,4-pyrone and 4,5-furan double bonds). The initial intercalation and interaction with double-stranded DNA occur after absorption of a photon of UVA and afterwards a pyrimidine residue of the DNA covalently binds to the first photoreactive site with a 5,6-double bond. The psoralen monoadducts formed in the DNA can further react photochemically with a pyrimidine base on the complementary strand of the DNA. In fact, it is precisely the planar structure of psoralens which allows them to intercalate between DNA bases[21], thus preventing cell mitosis (Figure 1). This feature is clinically relevant and the combination of psoralen and UVA irradiation (PUVA therapy) has been employed in autoimmune or hyper-proliferative skin diseases, including psoriasis and vitiligo[8]. However, extensive studies have pointed to other biological and clinical applications.

PSORALENS IN NORMAL AND TRANSFORMED CELL PHENOTYPES

Effects on normal cells

Diets rich in fruit and vegetables are often a predominant source of phytochemical compounds with defensive health effects. Initially, natural products were used as concentrated herbal extracts. However, the identification of the biological activity of each single component became a very complex matter due to a mixture of other constituents. In fact, herbal drugs or extracts contain a combination of active constituents, which are well known in popular medicine for the treatment of various kinds of disorders, such as asthma, coughs, nephritis, vitiligo, calvities and gastroenteric diseases. In Asian countries such as Mongolia, when people were infected with *Helicobacter pylori*, an alternative non-antibiotic method based on green tea catechins was found to strongly inhibit *H. pylori* urease activity *in vitro* and to suppress the bacterial-induced gastritis[22]. A few years later, in a cohort study of gastric carcinomas after screening a total of 25 food phytochemicals, bergamottin was designed as the most promising agent[12]. Grapefruit and grapefruit-based products are rich in flavonoids, coumarins and carotenoids, which in the long run have been shown to have anti-inflammatory[23], anticarcinogenic[9,10,24], antibacterial[12] activities and significant protective effects on cardiovascular diseases[9,13]. Furthermore, these bioactive compounds possess antioxidant properties because they act as free radical scavengers, therefore protecting cellular structures and functions in many stressful conditions.

A rich source of coumarins and coumarin containing compounds are the Psoralea corylifolia *L. seeds*, well known in traditional Chinese medicine as "Buguzhi". The plant has long been used for its *magical effects* to cure various skin diseases but, over the years, many other properties have been discovered[3,10]. The first historical notes on the biological effects of furanocoumarins are related to their photoactivation ability. As previously mentioned, PUVA has been suggested as a potential therapeutic to treat psoriatic lesions and other dermatological condi-

tions[25,26]. Studies reproduced in the 1980-90s on PUVA therapy for psoriasis have reported the comparison of oral and bathwater delivery of 8-MOP[27]. Compared with systemic administration, selectively bathing the epidermis with concentrated psoralen leads to a more complete reversal of the pathological epidermal alterations[11].

Psoriatic keratinocytes inappropriately synthesize a number of immune-related molecules and express a higher amount of epidermal growth factor receptors and insulin-like growth factor receptors that can well support the cellular hyperplasia of the psoriatic lesions. In fact, one of the first studies on PUVA demonstrated how this therapy strongly suppressed the mitogenic stimuli on keratinocytes[11].

A number of conditions with an autoimmune basis other than psoriasis, such as vitiligo, cutaneous T-cell lymphoma, pemphigus vulgaris, systemic sclerosis and rheumatoid arthritis, have benefited from the above treatment[28-30].

The compounds best known and widely used for these applications are 4,5',8-trimethylpsoralen (TMP), 8-MOP and 5-MOP. All these assessed in human cell line cultures *in vitro* as well as in *in vivo* studies showed antiproliferative activity and apoptotic effects. Along with other derivatives, two angular furanocoumarins angelicin and 4,6,4'-trimethyl angelin (TMA) in human keratinocytes photoinduce cellular death and cell cycle arrest in G1 phase. The molecular responses involve up-regulation of p21 $^{waf/Cip}$ and p53 activation, with mitochondrial-induced cytochrome *c* release and the consequent apoptotic reaction[31].

Effects on tumoral cells

In addition to these uses, coumarins display anticancer activities. Interest in this field stemmed from reports by Thornes who evidenced the immunomodulatory activity of coumarin and its utility in malignant melanoma[32]. The photoactivated coumarins are effective in preventing proliferation of bladder[33] and mucoepidermoid carcinoma[34], mammary cancer cell[35] and human melanoma cell line[36], with potential for their use in clinical treatments. Despite their photoactivity even in the absence of UV radiation, they have biological properties.

In fact, the native coumarins have been shown to affect adhesion and motility of neoplastic cells. This aspect was well elucidated in the highly invasive murine melanoma cell line B16-F10 by Velasco-Velaquez MA (2003).

In the latter cell type, compared to the non-malignant fibroblastic cells, the authors reported that 4-hydroxycoumarin (4-HC) was able to affect the assembly of actin filaments, thus decreasing the cellular adhesion to extracellular matrix proteins and motility only in the tumoral cell type.

Since adhesion of tumor cells to extracellular matrix is required during the metastatic process, 4-HC might be useful to prevent metastasis and could be used as an adjuvant therapy for melanoma[37].

The chemopreventive role of coumarin 5-MOP, in the absence of photoactivation, was investigated in human hepatocellular carcinoma (HCC) cell line by studying apoptotic and cytotoxic responses[38].

This study suggested that the suppressive effect of 5-MOP includes at least three modes of action: (1) it first kills cells directly; (2) induces apoptosis by arresting cells at the G_2/M phase of cell cycle; and (3) induces apoptosis through an independent pathway with cell-cycle arrest. The authors concluded that the inhibition of cyclin B1 by 5-MOP may play an important role in mitotic arrest and provide an additional way to prevent cells from entering the M phase and undergoing apoptosis.

Antitumoral activity of the methanolic seed extract of P corylifolia L was evaluated in two human cancer cell lines: oral carcinoma cell line and erythroleukemia cells and their corresponding multidrug-resistant cell lines. Both psoralen and isopsoralen constituents were able to inhibit the growth of these cells in a dose-dependent manner. They can also inhibit the growth of normal human primary cells but the IC_{50} values were higher than those of tumoral cells, suggesting that these two active components had potential selective cytotoxicity[10].

The resistance aspect of cancer cells to chemotherapeutic agents is one of the major obstacles in achieving an effective treatment for cancer. This results from a variety of factors, including individual variations in patients and somatic genetic differences in tumors. The most common reason for cancer drug resistance involves overexpression of membrane drug efflux pumps, such as P-glycoprotein, but other mechanisms might be implicated. Various Chinese herbal drugs have been evaluated for their specific actions against multi drug resistant (MDR) cancer cells. In an experimental study by Wu JYC of the University of Hong Kong[39], a bioassay-guided fractionation of extracts from Radix Peucedani (also known as "Baihua Qianhu" in Chinese medicine) led to the isolation of the pyranocoumarin compounds, (±)-3'-angeloyl-4'-acetoxy-cis-khellactone, a good candidate as a MDR reversing agent for tumoral cells. Strong synergistic interactions were demonstrated when pyranocoumarins were combined with common anti-tumor drugs, including doxorubicin, paclitaxel, puromycin or vincristine, in multidrug resistant human oral epidermoid carcinoma cell line (MDR KB-V1) compared to its drug-sensitive cell line, KB-3-1. Pyranocoumarins increased doxorubicin accumulation in KB-V1 cells and the same treatment down-regulated the expression of P-glycoprotein.

BREAST CANCER CELLS

Estrogen receptor status and estrogen/antiestrogen responsiveness

Estrogenic hormones are essential for mammary gland development but the same microenvironment plays a pivotal role in the initiation and progression of breast tumorigenesis. In addition, the weight of genetic factors that contribute to the development of breast cancer must be also taken into account.

Estrogen signaling pathways are mediated by two nuclear estrogen receptor (ER) proteins, ER-alpha and ER-beta, with different roles. ER-alpha transduces proliferative responses, thus determining cellular growth and tumor progression, while ER-beta has inhibitory actions[40-43].

Besides these nuclear receptors, the GPR30/GPER, a member of the seven-transmembrane G protein-coupled receptor family, has been implicated in mediating the effects of estrogens in various normal and cancer cells. In particular, GPER is able to trigger gene expression and proliferative responses induced by estrogens and even ER antagonists in hormone-sensitive tumor cells[44,45].

Indeed, a whole series of intracellular events, such as the rapid phosphorylation of mitogen-activated protein kinases (MAPK) ERK1/2, the activation of PI3-kinase (PI3K) and phospholipase C (PLC), the increase in cAMP concentrations and intracellular calcium mobilization, was shown to follow GPER activation by both estrogens and anti-estrogens[45].

Approximately 70% of breast cancers are ER-alpha positive and estrogen-dependent. However, the majority of these tumors will transit from an estrogen-dependent to an estrogen independent state that is usually associated with an aggressive form of the disease. During the initial stages of the disease, depletion of ER-alpha from breast cancer cells is a potent approach to prevent estrogen-dependent growth. For this reason, an anti-estrogen such as tamoxifen, acting as a competitive inhibitor of the receptor, has been implemented in the therapeutical protocols for breast cancer. However, the anti-hormonal long-term use of tamoxifen for most treated women leads to the development of drug resistance and an increase of endometrial cell proliferation with risk of endometrial carcinogenesis[46-48].

Therefore, the goal of minimizing the negative side effects of estrogens on breast tumor has stimulated the search for a new molecule able to block the agonistic effects of both estrogen and tamoxifen. The pure antiestrogen, Fulvestrant (ICI 182780), which competes with estrogen for binding to ER with a higher affinity, fulfils these properties very well. This drug is particularly effective as a second-line treatment when tumor cells develop resistance to tamoxifen[49].

In addition to blocking ER activity, scientific research has recently focused attention on the possible use of the aromatase inhibitors (AIs) since breast cancer cell growth is closely supported by estrogen production. Steroidal (exemestane) and non-steroidal (anastrozole, letrozole) aromatase inhibitors are an additional strategy to counteract estrogen function and signaling. These compounds either bind and inactivate aromatase or compete with endogenous substrates to reduce estrogen synthesis. They are approved for use in endocrine treatment of postmenopausal breast cancer and have demonstrated efficacy in patients that develop resistance to anti-hormonal therapy[50-52].

Several models have been proposed to explain the transition of breast tumor from an estrogen-dependent to an estrogen-independent status, including expression of variant or mutated ER-alpha, altered expression of co-factors or downstream estrogen target genes, post-receptor and pharmacological alterations[53] as well as ligand-independent activation of ER-alpha by other signaling pathways.

ER and growth factors

Accumulated evidence has indicated that a constitutive expression of growth factor or growth factor receptors in breast cancer cells plays an important role in pharmacological resistance. In many cases, an over-production of polypeptide growth factor with an increased activation of the corresponding signaling pathways can bypass the requirement of mitogenic estrogen signaling during progression of breast cancer[54].

Intricate interactions between ER-alpha and polypeptide growth factors, such as IGF-I, epidermal growth factor (EGF), transforming growth factor (TGF)-alpha and TGF-beta, are involved in the maintenance of proliferation and survival signals. In ER positive breast cancer cells, estrogens increase the mitogenic potential of IGF-I, sensitizing the cells to IGF-I action through the amplification of IGF-I signal[55-59].

The mechanisms of ER-alpha/IGF-I crosstalk is bidirectional and includes the ligand-independent activation of ER-alpha by IGF-I as well as the regulation of the IGF-I system by ER-alpha[60,61]. Other than this type of interaction, the crosstalk between ER-alpha and members of the EGFR family is well known. This receptor was found to be amplified in a human breast cancer cell line and named human epidermal growth factor receptor 2 (HER2)[62].

Amplification of HER2 in human mammary epithelial cells induces proliferative advantages, transformed characteristics, tumorigenic growth and in 3D models induces proliferative and anti-apoptotic changes that mimic early stages of epithelial cell transformation[63,64]. Overexpression of the HER2 protein, either through gene amplification or transcriptional deregulation, is seen in approximately 25%-30% of breast and ovarian cancers and confers worse biological behavior[65].

Patients with these characteristics have lower ER levels and are modestly less responsive to anti-estrogens; therefore, they develop the hormone-resistant phenotype. Moreover, high levels of HER-2/*neu* expression constitutively activate survival signals involving PI3K/Akt, which is closely related to MAPK hyperactivity[62,66].

Tyrosine kinase associated receptors control most of the fundamental cellular processes, including cell proliferation, differentiation, metabolism, migration and survival. The over-expression of these signals facilitates the emergence of anti-hormone resistance in breast cancer. In such cases, potential interventions with anti-growth factor agents, either alone or in combination with anti-estrogen agents, have been reported and have shown promising results[67].

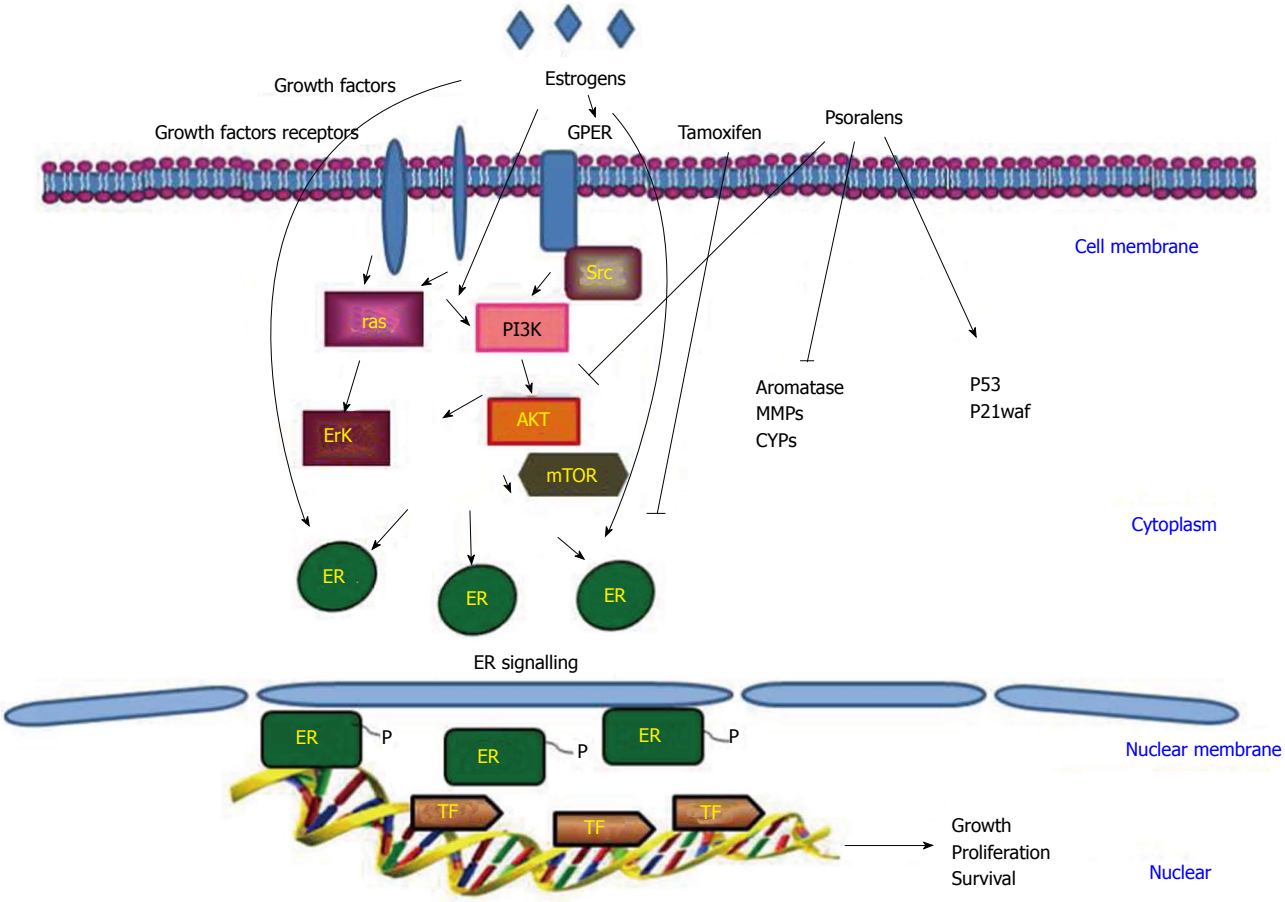

Figure 2 Estrogens and growth factor signaling in breast cancer. Mitogenic signals from estrogens and growth factors activate PI3K/AKT-Ras-Erk pathway to target estrogen receptor (ER). The phosphorylation cascade promotes ER activation. The receptor is recruited to a transcription factor (TF) that binds to responsive elements on DNA for the corresponding transcriptional responses. Antagonistic actions of tamoxifen on ER are shown. Psoralens may act by inhibiting aromatase enzyme, metalloproteinases (MMPs) and CYP enzymes. They address antagonistic effects on PI3K/AKT survival signals and apoptotic response with the involvement of p53 and p21 waf. Estrogens also bind the GPER, a member of the seven-transmembrane G protein-coupled receptor family, to trigger proliferative responses.

Targeting multiple pathways simultaneously may help to kill cancer cells and onset of slow drug resistance. In addition, the association of chemotherapy or radiotherapy with pure or synthetic analogs of phytochemical drugs may take advantage of the synergic effects of the combined protocols, resulting in the possibility of lower doses, consequently reducing toxicity. An overall view of the main signal transductions active in breast cancer is shown in Figure 2.

PSORALENS AND BREAST CANCER

Breast cancer signals and psoralen influence

A large number of epidemiological studies suggest that a daily intake of phytochemicals can reduce the incidence of several types of cancers, including breast tumors[68-71]. Moreover, genetic variation in pathways affecting absorption, metabolism and distribution of these natural substances can influence exposure at the tissue level, thus modifying disease risk in individuals[72,73].

The increasing research on this has revealed that the antiproliferative action of psoralens in many tumoral cells, as well as in breast carcinoma, is not only due to their photoactivation, but that these molecules exert their responses even in the absence of radiation. The biological activities in target tissues have been related to the binding of psoralens with specific receptor proteins identified in cytoplasmic and membrane fractions of responsive cells. Binding of psoralens to these proteins is of high affinity and reversible[74]. Coumarins and coumarin-related compounds have been reported to possess significant growth inhibitory activities in *in vivo* models and against a panel of breast cancer cell lines, in which the structure-activity relationships has also been evaluated[75-77].

Until a few years ago, the transductional pathways activated by psoralens in target cells were not well known; however, the growing interest on this aspect led to the identification of main signals by which the anti-tumoral action is exerted. Moreover, in our first study[78] it was demonstrated that bergapten "*per se*" without photoactivation was able to influence transductional pathways mainly involved in the regulation of cell survival in two hormone-dependent and hormone-independent human mammary tumoral cell lines, expression of the two biological variants of breast cancer respectively, MCF-7 and SKBR-3. The psoralen induced growth inhibition and

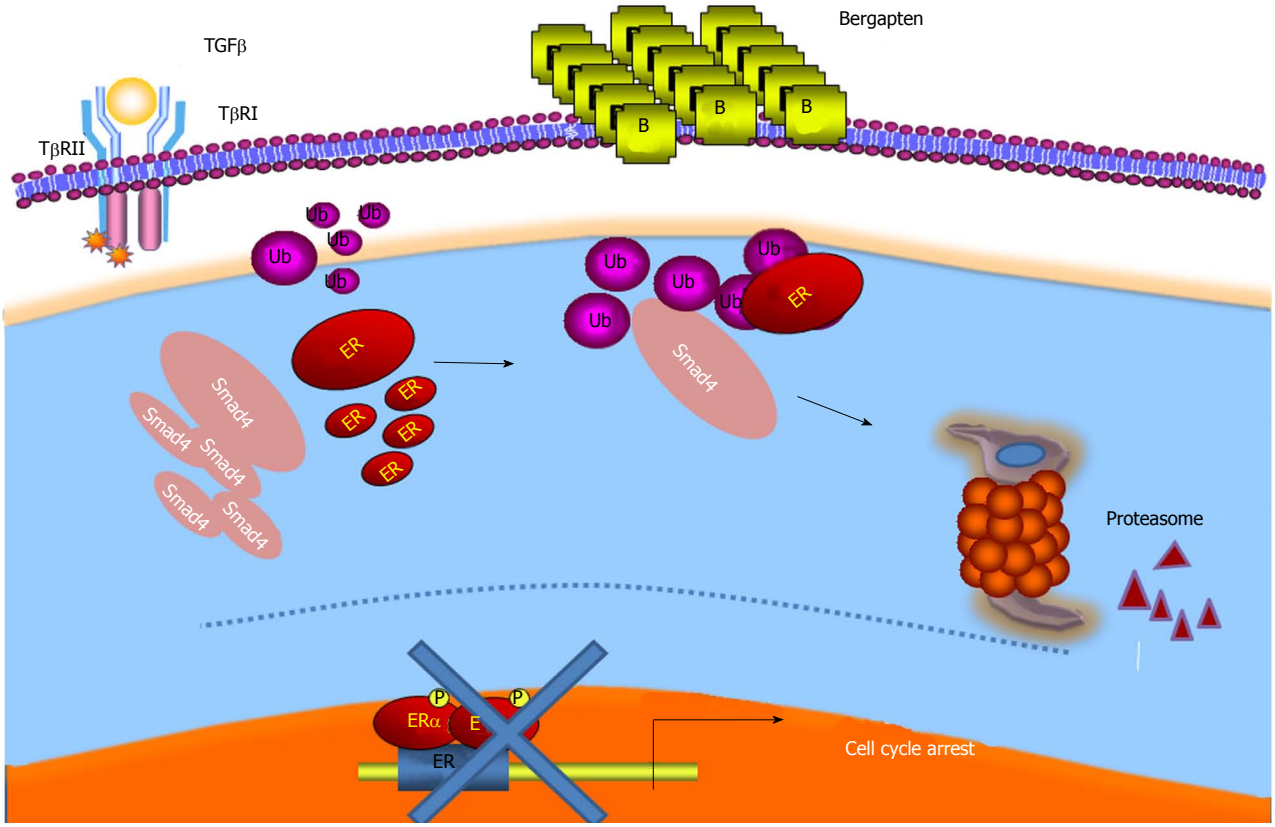

Figure 3 Bergapten affects estrogen receptor stability in breast cancer cells. Mechanism through bergapten (B) in breast cancer cells induces the polyubiquitination (Ub) of estrogen receptor (ER) with the involvement of transforming growth factor-β/SMAD4 protein. Immunoprecipitation assay performed in MCF-7 cells revealed that ER/SMAD4 and polyubiquitin are co-associated. The psoralen enhances the amount of SMAD4 and Poly-Ub complexed to ER, thereby regulating breast cancer cell progression[87].

apoptosis through the up-regulation of the cyclin inhibitor p21 waf and p53 mRNA and proteins. The molecular study addressed how bergapten transactivated p53 gene promoter through the involvement of the NF-Y nuclear transcriptional factor and the p38 MAPK activation.

Besides this, in hormone-dependent MCF-7 cells, the psoralen counteracted the stimulatory effects of two important mitogenic factors, estradiol and IGF-I on the PI3 kinase/Akt survival pathway.

Similarly, in the presence of photoactivation, bergapten preferentially addressed apoptosis at lower doses than those reported in the previous paper, as revealed by the increase of p53, caspase activation and DNA ladder, while in the absence of UV, the psoralen significantly reduced the p-Akt survival signal[79]. In estrogen-receptor positive breast cancer cells, as previously mentioned, estradiol exerts its main role supporting the proliferation and growth of mammary tissue.

Aromatase is the enzyme responsible for the conversion of androgens into estrogens and synthetic aromatase inhibitors, such as letrozole, anastrozole and exemestane, have proven to be effective in endocrine regimens for ER-positive breast cancer. Together with these molecules, several flavones have also been demonstrated to be effective inhibitors of aromatase and NADPH-quinone reductase 1 and 2 both play an important role on breast carcinogenesis. Among the coumarin-derived compounds, the 4-benzyl-3-(4'-chlorophenyl)-7-methoxycoumarin has been shown to be a potent aromatase inhibitor. The structure-activity studies have evidenced that the three functional groups of the coumarin [the 3-(4'-chlorophenyl), 4-benzyl, and 7-methoxyl groups] are important for its ability to inhibit aromatase[72,80] and not only this (Figure 2). The realization that, in addition to the formation of estrogens by the aromatase pathway, steroids with estrogenic properties could also be formed *via* a sulfatase route has stimulated the interest of other authors in developing potent steroid sulfatase (STS) inhibitors. The furanocoumarins have proved to be successful steroid sulfatase inhibitors once tested in breast cancer cells and for this they may be useful in suppressing estrogen-dependent breast tumors[72,81,82]. The development and testing of both aromatase and sulfatase inhibitors are in progress and should resolve the question as to whether inhibition of only aromatase or sulfatase is superior to inhibition of only aromatase or STS activity when used for the treatment of hormone-dependent breast cancer.

Several observations have also documented the interplay between E2/ER and growth factor signals such as the TGF-beta-dependent pathway. Indeed, it has been

evidenced that ER-alpha is able to physically interact with components of the latter pathway, SMAD2, SMAD3 and SMAD4, and to abrogate TGF-beta signaling cascade[83,84]. On the other hand, while TGF-beta signaling has been demonstrated to stimulate ER-alpha transcriptional activity, the complex of SMAD3 and SMAD4 inhibits its activity[85,86]. Analogously, treatment of breast cancer-sensitive and tamoxifen-resistant cells with bergapten induced a depletion of ER protein through a degradative process that sees the involvement of the SMAD4 protein[87] (Figure 3).

This study once again draws attention to the anti-tumoral properties of psoralen and highlights a new molecular mechanism through which bergapten may prevent the crosstalk between the receptor and growth factor mitogenic signaling by affecting ER-alpha stability in breast cancer tamoxifen-sensitive and resistant cells. However, in a recent paper[88], it was demonstrated well that psoralen can also affect the Erb2 receptor tyrosine kinase whose over-expression, as previously reported, characterizes the most aggressive forms of breast cancer.

Independently of interstrand DNA crosslinks, the photo-activated 8-MOP interacts with the Erb2 catalytic autokinase domain, blocking its activity, and furthermore, it can reverse therapeutic resistance by triggering tumor cell apoptosis.

Psoralen action on pro-metastatic and detoxification enzymes

A further negative aspect of estrogens is to promote the migration and motility of breast cancer cells, as demonstrated in an "*in vitro*" assay, and in fact, they increase the closure of wounded confluent culture. This phenomenon is dependent on the expression of ER, because antiestrogens completely abolish the migratory potency of estrogens[89]. A number of proteolytic enzymes participate in the degradation of environmental barriers, such as the cell-extracellular matrix (ECM) and basement membrane. Matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases, play an important role in the proteolysis of ECM components, thus supporting metastatic and angiogenetic processes[90-92].

Agents able to suppress MMP signaling are useful to target cancer metastasis. Some natural products have been demonstrated to play a remarkable role in inhibiting MMP enzymes in breast and other type of cancers[93-97].

The antimetastatic activity of furanocoumarin bergamottin has been described in human fibrosarcoma HT-1080 cells and in MCF-7 breast cancer cells where the compound suppresses MMP-9 gene expression by repressing the transcriptional activation in the MMP-9 promoter. The results obtained in this study evidenced that the furanocoumarin inhibited PMA or TNF-alpha-induced activation of MMP-9 by suppressing NFκB activation in tumoral cells[98].

Considering that psoralen influences bone metabolism and that breast cancer frequently metastasizes to the skeleton, one study[99] investigated whether it can affect this process *in vivo*. Histological, molecular, biological and imaging analyses revealed that psoralen inhibits bone metastasis; in fact, it regulated the function of osteoblasts and osteoclasts in tumor-bearing mice. Accordingly, the authors suggest the possible therapeutic role of the drug for metastatic breast cancer.

In addition to the above mentioned effects, the furanocoumarin bergamottin has also been described to inhibit members of the family of CYP enzymes (cytochrome P450s) involved in the metabolism of many xenobiotics and drugs. It is well established that tumorigenesis is closely linked to the metabolism of pro-carcinogenic substances, which when subjected to biotransformation into the cells become dangerous for the body. Certain linear furanocoumarins (*e.g.*, bergamottin, imperatorin, isopimpinellin) and a simple coumarin (osthruthin) were found to inhibit cytochrome P450 and to reduce the formation of DNA adducts induced by benzo[a]pyrene and 7,12-dimethylbenz[a] anthracene[24,100-103]. This evidence has encouraged more studies in which five new tetracyclic benzofurocoumarins were synthesized and then tested in three different human tumor cell lines: MDA MB 231 (breast adenocarcinoma), HeLa (cervix adenocarcinoma) and TCC-SUP (bladder transitional cell carcinoma). All of them significantly inhibited cell proliferation and this was mainly linked with the inhibition of CYP2A6 enzyme, belonging to the family of CYPs. The effectiveness of the drugs is related to the chelation of the oxygen from the furan ring with the iron from the heme, possibly resulting in the inactivation of the enzyme, and this might be one of the main causes that preclude tumor cell proliferation[104].

CONCLUSION

Currently, there is a great scientific interest towards natural anticancer drugs due to their multiple target activities on tumoral cells. As reported here, from the early studies on the activity of psoralens, much has now been documented, especially with regards to their mechanism of action at the molecular level.

The anti-tumoral activity of these molecules against breast cancer has been the main point reported in this review. Many intracellular signals that maintain high survival of breast cancer cells are selectively affected by these drugs. Starting from the latest experimental investigations, it appears that even the most aggressive and resistant cell phenotypes are responsive to psoralens since they antagonize metabolic pathways, protease enzymes, cell cycle progression and even interfere in the crosstalk between receptors and growth factor mitogenic signaling. The combination of natural products with the traditional chemotherapeutic agents, with the purpose of using low doses, can be well addressed and may be a new opportunity for the treatment of breast tumors, thereby decreasing the side effects at the systemic level.

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