

Pterostilbene in Cancer Therapy

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Natural polyphenols are organic chemicals which contain phenol units in their structures and possess antitumor properties. However, a key problem is their short half-life and low bioavailability under in vivo conditions. Pterostilbene (3,5-dimethoxy-4'-hydroxystilbene; PT) is a phytoalexin originally isolated from the heartwood of red sandalwood. As recently reported by our group, PT was shown to be effective in the treatment of melanoma. Counterintuitively, PT is not effective (cytotoxic) against melanoma in vitro, and only under in vivo conditions does PT display its anticancer activity. This study elucidated that PT can be effective against melanoma through the inhibition of adrenocorticotrophic hormone production in the brain of a mouse, which weakens the Nrf2-dependent antioxidant defenses of melanoma and also pancreatic cancers. This results in both the inhibition of tumor growth and sensitization of the tumor to oxidative stress. Moreover, PT can promote cancer cell death via a mechanism involving lysosomal membrane permeabilization. Different grades of susceptibility were observed among the different cancer cells depending on their lysosomal heat shock protein 70 content, a known stabilizer of lysosomal membranes. In addition, the safety of PT administered i.v. has been evaluated in mice. PT was found to be pharmacologically safe because it showed no organ-specific or systemic toxicity (including tissue histopathologic examination and regular hematology and clinical chemistry data) even when administered i.v. at a high dose (30 mg/kg per day × 23 days). Moreover, new pharmacological advances are being developed to increase its bioavailability and, thereby, its bioefficacy. Therefore, although applications of PT in cancer therapy are just beginning to be explored, it represents a potential (and effective) adjuvant/sensitizing therapy which may improve the results of various oncotherapies. The aim of this review is to present and discuss the results that in our opinion best support the usefulness of PT in cancer therapy, making special emphasis on the in vivo evidence.

Keywords: pterostilbene ; polyphenols ; stilbenes ; cancer ; oxidative stress

1. Introduction

At present, combined therapies involving distinct molecular mechanisms are considered the most promising strategies for cancer treatment. Although such combinations have shown efficacy in preclinical models, the results in clinical trials have not been encouraging in many cases. This suggests that malignant cells (likely particular cell subsets), treated to block specific pathways, find ways to adapt using alternative survival mechanisms. Based on available experimental evidences, by increasing the efficacy of biotherapy, cytotoxic drugs, or ionizing radiation, and/or by directly promoting cancer cell death, pterostilbene (PT) may be a useful agent to treat different established cancers. This prompted us to update which evidence can support the potential use of PT in clinical oncotherapy.

PT (3,5-dimethoxy-4'-hydroxystilbene) is a polyphenol (compounds derived from the shikimate/phenylpropanoid and/or the polyketide pathway, featuring more than one phenolic unit and without nitrogen-based functional groups) as well as a phytoalexin (antimicrobial substance synthesized de novo by plants). PT is also the natural analog of resveratrol (3,5,4'-trihydroxystilbene), but is a much stronger antifungal agent (>10 times) than resveratrol. Moreover, PT, with two methoxy groups and one hydroxyl group, has greater lipophilicity and a higher potential for cellular uptake than resveratrol, which has three hydroxyl groups ^[1].

PT, a secondary metabolite of plants originally isolated from the heartwood of red sandalwood (*PTocarpus santalinus*), is also found in other plants and berries, e.g., blueberries (approx. 10–15 mg/kg of fresh weight) ^{[2][3]}. A resveratrol O-methyltransferase, which catalyzes the synthesis of PT from resveratrol, was identified in grapevine leaves where it is induced by different types of stress ^[4]. Indeed, PT is involved (as are other natural polyphenols) in plant defense against different stressful conditions, i.e., UV radiation, aggression by pathogens, low soil fertility, high/low temperatures, severe drought, or grazing pressure ^{[5][6]}.

Based on reported experimental evidence, PT has potential health benefits in inflammatory dermatoses, photoprotection, cancer prevention and therapy, insulin sensitivity, blood glycemia and lipid levels, cardiovascular diseases, aging, and memory and cognition ^{[7][8]}.

Cancer cells exhibit high levels of oxidative stress, as compared to their normal counterparts [9]. A rise in intracellular reactive oxygen species (ROS) levels has two potentially important effects: damage to various cell components and triggering of the activation of specific signalling pathways. Both effects can influence numerous cellular processes linked to cancer progression. ROS have been shown to promote the proliferation of cancer cells, which highlights their cancer promoting potential. The exposure of several cancer cell lines to inflammation- or chemically induced ROS boosts their migratory and invasive behaviors, hence suggesting a role of these reactive species in favoring the invasive phenotype. Besides, it is well known that exposure to ROS above a certain threshold irreversibly leads to cell damage, and eventually to cell death [10][11][12]. Therefore, the net result of pro- and anti-cancer ROS effects may likely determine the rate and extent of in vivo tumor progression. Based on this reasoning, elevated rates of intrinsic reactive oxygen species (ROS) generation may confer a higher susceptibility of cancer cells to further oxidative stress induced by treatments. However, due to the innate characteristics of in vivo growing cancers, this strategy could facilitate the selection of cancer cell subclones highly resistant to therapies. In this scenario, increased ROS generation could contribute to the ability of cancers to mutate, injure local tissues, and promote tumor heterogeneity and metastases [13]. Paradoxically, by reducing the redox stress in cancer cells, antioxidant supplements (and pharmaceuticals) could decrease the effectiveness of radiotherapy and chemotherapy. However, as reported by our group, PT was shown to be effective in the treatment of malignant melanoma. Counterintuitively, PT was not found effective (cytotoxic) in vitro, only in vivo. PT can be effective against melanoma by decreasing adrenocorticotropin hormone (ACTH), which interferes in the Nrf2-dependent activity in the metastatic cells [14]. Nrf2 is a transcription factor that regulates the expression of antioxidant and defense proteins that protect against oxidative damage triggered by injury and inflammation [14]. In fact, in many cancer cells the Keap1-mediated Nrf2 downregulation is abolished or diminished, thus generating an addiction to Nrf2 and, thereby, favoring cancer cell defenses and progression [15].

2. Metabolism and Pharmacokinetics

Regarding potential applications of PT in cancer therapy, its administration (depending on the indication) can be carried out orally, i.v., or topically.

Orally administered polyphenols, including PT, undergo rapid conjugation in the intestinal tract of humans and rodents. Polyphenol aglycones, when absorbed by intestinal enterocytes, undergo extensive phase II metabolism via uridine 5'-diphospho-glucuronosyltransferase isoforms. Conjugates are absorbed with very little of the free chemical structure gaining access to the systemic blood circulation. Moreover, polyphenol aglycones present in the blood undergo further (and also rapid) metabolism in the liver to methylated, glucuronidated, and/or sulfated conjugates. These features, as well as the mechanisms of elimination in the urine and via the biliary tract, as well as recycling by the intestinal tract, have been reviewed in detail (e.g., Estrela 2013, Liu 2020) [7][8].

Available data strongly suggest that natural polyphenols and their derivatives are biologically more active than their metabolites. Therefore, orally administered polyphenols are unlikely to be systemically effective unless their biological effects are not inactivated by conjugation, and/or the free polyphenol can be released by the hydrolysis of conjugates and taken up by cancer cells in sufficient amounts to generate pharmacologically active concentrations. These limitations may be minimized in the case of primary tumors of the gastrointestinal tract where the orally administered polyphenol can directly reach the growing tumor.

Alternatively, PT can be administered i.v. When 20 mg/kg of PT was administered i.v. to mice, pharmacokinetics studies showed a peak of approximately 95 μ M in plasma 5 min after administration, which then rapidly decreased to about 20 μ M at 60 min and 2 μ M at 240 min. Potential recycling did not further increase PT plasma levels (approx. 1 μ M 480 min after i.v. administration) [16]. Plasma and total blood levels were not significantly different [16]. Tumor levels of PT, following i.v. administration, were even lower. For example, after the i.v. administration of PT at 30 mg/kg, the highest concentration range observed in the plasma of nude mice bearing different human melanomas was 98–116 μ M 5min after administration. These levels decreased rapidly to approximately 1 μ M at 480 min. PT levels in tumors were measured in parallel and also reached the highest concentration (28–33 μ M) 5 min after administration, whereas the lowest concentration (~1 μ M) was measured at 180 min. The half-life of PT in in vivo growing melanoma tumors was of 36–40 min [14]. These experimental data clearly exemplify the limitations of bioavailability that PT has (and polyphenols in general) and that temper its potential efficacy under in vivo conditions.

The topical administration of PT to cutaneous cancers, located in the skin and/or subcutaneous tissues, does not have the bioavailability problems mentioned above. Skin keratinocytes have different phase I (reduction/oxidation) and II (biotransformation) enzyme activities [17][18]. Accordingly, we found that topically administered PT (using liposomes as

carriers) completely prevents chronic UVB-induced skin carcinogenesis [19]. This anticarcinogenic effect was associated with the maintenance of skin antioxidant defenses and the inhibition of UVB-induced oxidative damage [19]. Therefore, the anticancer activity of PT did not depend on direct antioxidant activity of the molecule itself.

3. Toxicity

In Swiss mice fed with a PT-enriched diet at doses of 0, 30, 300, and 3000 mg/kg body weight/day red blood cell number and the hematocrit increased (approx. 25%) compared to control mice, whereas biochemical parameters were not significantly affected. Histopathology, hematology, clinical chemistry, and urinary balance studies found no alterations induced by PT as compared to controls [20], thus concluding that orally administered PT, even at the highest dose administered, was nontoxic. More recent studies suggest a no-observed-adverse-effect-level (NOAEL) of 200 mg of 3'-hydroxy-pterostilbene (a natural PT analog)/kg body weight/day in rats after oral administration [21].

At present, there are 13 registered clinical trials under www.clinicaltrials.gov (accessed on 14 February 2021) where PT has been used in humans, alone or in combination with other compounds. Based on the available data, orally administered PT appears safe at a dose of 125 mg twice daily (NCT01267227) [22]. Safety concerns forced a previous trial to stop, where the safety and activity of SRT501 (a formulation of resveratrol claimed to increase its in vivo bioavailability), alone or in combination with bortezomib, were being evaluated (NCT00920556). In this trial, where 5g of SRT501/day was administered orally for 20 consecutive days, kidney damage developed in some patients, thus raising the question of whether high doses (>1 g/day), administered chronically could pose toxicity problems.

Intravenous administration has been also evaluated in nude mice. PT was found to be pharmacologically safe (no organ-specific or systemic toxicity, i.e., tissue histopathologic examination and regular hematology and clinical chemistry data) even when administered i.v. (dissolved in DMSO:ethanol [2:1]) at a dose of 30 mg/kg per day × 23 days [23], or at a dose of 40 mg/kg every 48h × 5 weeks [14]. The i.v. administration has never been tried in humans. However, the availability of a hydrosoluble disodium salt of PT phosphate (e.g., www.lgcstandards.com, accessed on 14 February 2021) may facilitate its use in cancer.

4. Pharmaceutical Formulations, Structural Modifications, and Delivery Systems

As it is in general the case for all natural polyphenols, the low systemic bioavailability of PT limits its anticancer potential. Consequently, methods to improve its absorption rate and pharmacokinetics, and thereby its bioavailability, would positively affect its therapeutic efficacy. On this issue and regarding polyphenols in general, different options have been recently discussed (see, e.g., Estrela 2017) [24]. Potential options to increase PT bioavailability may include:

- (a) Prodrugs (i.e., carboxyesters, sulfonates, sulfates, phosphates, acetals, carbamates, and carbonates). The availability of a phosphorylated salt of PT, which increases its polarity and water solubility, has been mentioned above). Prodrugs in which the hydroxyl moiety is reversibly protected as a carbamate ester linked to the N-terminus of a natural amino acid (isoleucine or β -alanine) afforded increased absorption, reduced metabolism and higher concentrations of PT, sustained for several hours, in different organs [25]. Bis(hydroxymethyl)propionate analogs of PT have shown high anticancer activity against cisplatin-resistant human oral cancer cells [26].
- (b) Solubilizing the compound in an organic solvent and subsequently adding it into an aqueous phase that contains a suitable stabilizer results in an emulsion. The homogenization of the emulsion and dilution in water may favor the precipitation of uniform nanoparticles. For instance, this methodology increases, e.g., curcumin bioavailability several fold [27]. In nanoemulsions, the higher unsaturation levels of lipids improved the lipid digestibility and PT bioaccessibility [28].
- (c) Polyionic/polymeric shells encapsulating nanoparticles, solid lipid nanoparticles, or the conjugation of nanoparticles with ligands such as folic acid (which may recognize specific cell surface molecules in target cells), are additional options [29]. Moreover, an antibody-4arm-polyethylene glycol-PT conjugate has been synthesized for the targeted co-delivery of anticancer drugs to solid tumors [30]. Zein/fucoidan nanoparticles are a promising delivery carrier for the encapsulation, protection, and release of PT [31]. Whereas poly(2-oxazoline)-PT block copolymer nanoparticles, where a poly(2-methylsuccinate-2-oxazoline) segment conjugates PT, can be also used for dual anticancer drug delivery [32].
- (d) Liposomes. For instance, lipophilic 3-oxo-C(12)-homoserine lactone and stilbene derivatives can be loaded into liposomal lipid bilayer with efficiencies of 50–70% [33]. Liposome-engulfed PT was highly efficient for the topical administration of PT [19]. Nevertheless, it has not been assayed for parenteral administration yet.
- (e) Implantable drug delivery systems, such as Alzet-like reservoir pumps (ALZA/Durect Corp., Cupertino, CA) (controlled-release drug delivery which use osmotic gradients generated after their subcutaneous implantation); or matrix-type

implants, which entrap the drug in a polymeric matrix and can provide high local concentrations of the drug and/or release it slowly into the blood circulation [34].

(f) Exosomes represent good vehicles due to their biocompatibility, stability in blood circulation, and even ability to target them to certain cell and tissue types. These carriers have already been used for other polyphenols [24], and its use has been suggested for PT [35].

(g) Cocrystals. The propensity of PT to form cocrystalline materials with active pharmaceutical ingredients was first studied by Schultheiss et al. [36], who found that the caffeine cocrystal solubility was 27× higher than the PT solubility. The same authors also reported the cocrystallization of PT with carbamazepine [37]. More recent advances are under development (e.g., www.circecrystal.com, accessed on 14 February 2021).

5. Anticancer Effects and Mechanisms

Here, we will summarize published reports that have examined the anticancer effects of PT under in vitro (Table 1) and in vivo (Table 2) conditions. In order for a publication to be included in Table 1, and reviewed here, a minimum of two cell lines needed to be examined unless PT was being examined in combination with another molecule. PT has garnered increasing interest from cancer researchers over the last decade as a natural molecule with anticancer properties that has been shown to be safe for human use (see above).

Table 1. PT and cancer cells under in vitro conditions: effects and proposed mechanisms. NSCLC: non-small cell lung cancer.

Cancer Type	Concentration(s) Analyzed	Time of Incubation (hours)	Anticancer Effect	Proposed Mechanism	Reference
Lung	PT (10 µM) + Osimertinib (0.02 µM)	24	Synergistic anticancer effect against two EGFR- mutation positive NSCLC cells	The combination reversed osimertinib- induced STAT3 activation and suppressed src activation	[38]
Cervical	PT (20 and 40 µM)	48	Inhibition of growth and metastatic ability of both adherent and stem-like cancer cells	Induction of ROS- induced apoptosis and inhibition of MMP 2/9 expression	[39]
Pancreatic	PT (50 and 75 µM)	72	Induced cell cycle arrest and apoptosis in Gemcitabine- resistant cancer cells	Inhibitions of multidrug resistance protein (MDR1) expression via reduction in Akt signaling	[40]
Ovarian	PT (18.5 to 300 µM) +/- Cisplatin (3.125 to 50 µM)	48	Induction of cell cycle arrest and apoptosis against several ovarian cancer cell lines and synergy with cisplatin	Downregulation of JAK/STAT3 pathway	[41]
Oral	PT (50 and 75 µM)	24 or 48	Induction of apoptosis of cisplatin-resistant oral cancer cells	Activation of intrinsic apoptosis cascade and downregulation of MDR1	[42]

Cancer Type	Concentration(s) Analyzed	Time of Incubation (hours)	Anticancer Effect	Proposed Mechanism	Reference
Breast	PT (2.5 to 10 μ M)	24	Upregulation of apoptotic pathways in two mutant-p53 cell lines	Induction of pro-apoptotic Bax protein and caspase-3 activity. Decreased mutant p53 protein	[43]
Breast	PT (10 and 20 μ M) + Tamoxifen (5 μ M)	24	PT + Tamoxifen showed an additive inhibitory effect on breast cancer cells	Increased apoptosis	[44]
Gastrointestinal	PT (10 and 100 μ M)	48	PT showed dose-dependent inhibition of cell proliferation in three GI cancer cell lines	Increase in mitochondrial membrane potential, ROS and lipid peroxide	[45]
Prostate	PT (10 to 100 μ M)	48	PT showed dose-dependent inhibition of cellular proliferation in three prostate cancer cell lines	Activation of AMPK	[46]
Pancreatic	PT (10 to 100 μ M)	72	PT is cytotoxic against two pancreatic cancer cell lines.	Inhibition of cell proliferation and/or cell death, mitochondrial membrane depolarization and activation of caspases.	[47]
Melanoma, colon, breast, and lung	PT (10 to 50 μ M)	72	PT demonstrates differential toxicity to various cancer cell lines	PT is more efficacious in melanoma and lung cancer cells that have low HSP70 expression than in high HSP70 colon and breast cancer cells	[48]

Table 2. PT and cancer: in vivo evidences. AOM: azoxymethane; HCC: hepatocellular carcinoma; TPA: 12-O-tetradecanoylphorbol-13-acetate.

Cancer Type	Concentration(s) Analyzed	Administration	Anticancer Effect	Proposed Mechanism	Reference
Cervical	PT (1 mM)	Intralesional injection daily for 5 days	PT inhibits tumor development in HPV E6-positive cervical cancer mouse model	Decrease in tumor size due to increase in apoptosis, and downregulation of E6 and VEGF tumor protein levels	[49]
Breast	PT (40 µg/kg) + Vitamin E (42 IU/kg or 99 IU/kg)	PT oral 3 times per week Vit E in diet	PT and vit E inhibited breast tumor growth and invasion in mouse xenograft model	Inhibition of Akt and downregulation of cell cycle proteins	[50]
Breast	PT (56 mg/kg every 4 days for 3 weeks)	Oral gavage	PT induces apoptosis and inhibits tumor growth of ER-Breast cancer xenograft model	Inhibition of ER-α36 (a variant of full-length Estrogen receptor) resulting in inhibition of Akt signaling	[51]
Prostate	PT (50 mg/kg)	Intraperitoneal Injections daily (5 days/week) for 39 days	PT reduced tumor growth in mouse xenograft model	Downregulation of miR-17-5p and miR-106-5p expression in both tumors and circulation	[52]
Breast	PT (10 mg/kg)	Intraperitoneal injections 3 times a week	PT suppressed tumor growth and metastasis in xenograft mouse model	Reduction in src signaling and inhibition of EMT	[53]
Pancreatic	PT (100 µg/kg, 500 µg/kg or 1 mg/kg)	Oral gavage	PT inhibited tumor growth rates	Increases MnSOD antioxidant activity; inhibits STAT3 activity	[54]
Melanoma	PT (30 mg/kg) every 48 h for 5 weeks	Intravenous	PT decreased tumor growth in mouse xenograft model	Downregulated adrenocorticotropin hormone (ACTH) resulting in decrease Nrf2-mediated antioxidant defenses	[14]
Lymphoma	PT (30 mg/kg every 2 days for 20 days)	Intravenous	PT inhibited tumor growth in diffuse large B-cell lymphoma xenograft mouse model	Cytotoxic effect due to reduction in mitochondrial membrane potential and increase in apoptosis and ROS levels	[55]

Cancer Type	Concentration(s) Analyzed	Administration	Anticancer Effect	Proposed Mechanism	Reference
Breast	PT (0.1% w/w in diet)	Oral	PT suppressed tumor growth in triple-negative breast cancer xenograft mouse model	Inhibition of Akt activation and upregulation of Bax	[56]
Prostate	PT (50 mg/kg/day)	Intraperitoneal	PT inhibited tumor growth and metastasis in prostate cancer xenografts	Reduction in metastasis-associated protein 1 (MTA1) and increased apoptosis	[57]
Endometrial	PT (30 mg/kg/day) + Megestrol acetate (10 mg/kg/day)	Oral gavage	PT synergizes with megestrol acetate for reduction of tumor growth in xenografts	Suppression of STAT3 activation as well as decreased ER expression	[58]
Biliary	PT (30 and 60 mg/kg every 2 days For 3 weeks)	Intraperitoneal	PT inhibited tumor growth in xenograft mouse model	Inhibited cell progression and induced autophagy	[59]
Multiple Myeloma	PT (50 mg/kg/day For 2 weeks)	Intraperitoneal	PT reduced tumor volume in mouse xenografts	Inhibited cell progression. Induction of apoptosis through increased ROS generation and activation of ERK1/2 and of JNK signaling	[60]
Colon	PT (40 ppm diet for 45 weeks)	Oral	PT reduced AOM-induced colon tumor multiplicity	Inhibits cell proliferation via reduced PCNA expression and reduced beta-catenin and cyclin D1. Reduction of inflammatory markers	[61]
Colorectal	PT (20 mg/kg/day) + quercetin (20 mg/kg/day)	Intravenous	PT + QUER inhibited tumor growth by 51% in xenografts	Increase in SOD2 expression and decrease in Bcl-2 expression	[23]
Liver	PT (100 and 200 mg/kg/day)	Intraperitoneal	PT dose-dependently inhibited HCC tumor growth in mouse model	Increase in p53 expression and ROS generation and activation of apoptosis	[62]

Cancer Type	Concentration(s) Analyzed	Administration	Anticancer Effect	Proposed Mechanism	Reference
Skin	PT (1-2 μ mol)	Topical	PT prevented UV-B induced skin cancer in mouse model	Maintenance of skin antioxidant defenses including Nrf2 activation	[19]
Skin	PT (1 and 5 μ mol)	Topical	PT suppressed TPA-induced skin cancer in mouse model	Downregulation of iNOS and COX-2	[63]
Glioblastoma Multiforme	PT (2 mg/kg, three times a week)	Intraperitoneal	PT suppressed tumorigenesis in glioma stem cell mouse xenograft	Inhibition of GRP78	[64]
Colon	PT (50 and 250 ppm in diet, 24 weeks)	Oral	PT prevents AOM-induced colon tumorigenesis.	Reduction of NF- κ B activation, as well as iNOS and COX-2 expression Activation of Nrf2 signaling	[65]
Melanoma	PT (20 mg/kg/day) + QUER (20 mg/kg/day)	Intravenous	PT + QUER shown to inhibit metastasis of melanoma in xenografts	Inhibition of Bcl-2	[16]

References

- Kim, H.; Seo, K.-H.; Yokoyama, W. Chemistry of Pterostilbene and Its Metabolic Effects. *J. Agric. Food Chem.* 2020, 68, 12836–12841.
- Spath, E.; Schlager, J. On the constituents of “Red Sandalwood” [*Pterocarpus santalinus*]. 2: The constitution of pterostilbene. *Ber. Dtsch. Chem. Gesellsch.* 1940, 73, 881–884.
- Tsai, H.-Y.; Ho, C.-T.; Chen, Y.-K. Biological Actions and Molecular Effects of Resveratrol, Pterostilbene, and 3'-Hydroxypterostilbene. *J. Food Drug Anal.* 2017, 25, 134–147.
- Schmidlin, L.; Poutaraud, A.; Claudel, P.; Mestre, P.; Prado, E.; Santos-Rosa, M.; Wiedemann-Merdinoglu, S.; Karst, F.; Merdinoglu, D.; Hugueney, P. A Stress-Inducible Resveratrol O-Methyltransferase Involved in the Biosynthesis of Pterostilbene in Grapevine. *Plant Physiol.* 2008, 148, 1630–1639.
- Chong, J.; Poutaraud, A.; Hugueney, P. Metabolism and Roles of Stilbenes in Plants. *Plant Sci.* 2009, 177, 143–155.
- Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouységou, L. Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. *Angew. Chem. Int. Ed. Engl.* 2011, 50, 586–621.
- Estrela, J.M.; Ortega, A.; Mena, S.; Rodriguez, M.L.; Asensi, M. Pterostilbene: Biomedical Applications. *Crit. Rev. Clin. Lab. Sci.* 2013, 50, 65–78.
- Liu, Y.; You, Y.; Lu, J.; Chen, X.; Yang, Z. Recent Advances in Synthesis, Bioactivity, and Pharmacokinetics of Pterostilbene, an Important Analog of Resveratrol. *Molecules* 2020, 25, 5166.
- Szatrowski, T.P.; Nathan, C.F. Production of Large Amounts of Hydrogen Peroxide by Human Tumor Cells. *Cancer Res.* 1991, 51, 794–798.

10. Ortega, A.L.; Mena, S.; Estrela, J.M. Oxidative and Nitrosative Stress in the Metastatic Microenvironment. *Cancers* 2010, 2, 274–304.
11. Gill, J.G.; Piskounova, E.; Morrison, S.J. Cancer, Oxidative Stress, and Metastasis. *Cold Spring Harb. Symp. Quant. Biol.* 2016, 81, 163–175.
12. Galadari, S.; Rahman, A.; Pallichankandy, S.; Thayyullathil, F. Reactive Oxygen Species and Cancer Paradox: To Promote or to Suppress? *Free Radic. Biol. Med.* 2017, 104, 144–164.
13. Gorrini, C.; Harris, I.S.; Mak, T.W. Modulation of Oxidative Stress as an Anticancer Strategy. *Nat. Rev. Drug Discov.* 2013, 12, 931–947.
14. Benlloch, M.; Obrador, E.; Valles, S.L.; Rodriguez, M.L.; Sirerol, J.A.; Alcácer, J.; Pellicer, J.A.; Salvador, R.; Cerdá, C.; Sáez, G.T.; et al. Pterostilbene Decreases the Antioxidant Defenses of Aggressive Cancer Cells In Vivo: A Physiological Glucocorticoids- and Nrf2-Dependent Mechanism. *Antioxid. Redox Signal.* 2016, 24, 974–990.
15. Okazaki, K.; Papagiannakopoulos, T.; Motohashi, H. Metabolic Features of Cancer Cells in NRF2 Addiction Status. *Biophys. Rev.* 2020, 12, 435–441.
16. Ferrer, P.; Asensi, M.; Segarra, R.; Ortega, A.; Benlloch, M.; Obrador, E.; Varea, M.T.; Asensio, G.; Jordá, L.; Estrela, J.M. Association between Pterostilbene and Quercetin Inhibits Metastatic Activity of B16 Melanoma. *Neoplasia* 2005, 7, 37–47.
17. Strohm, B.H.; Kulkarni, A.P. Peroxidase, an Alternate Pathway to Cytochrome P-450 for Xenobiotic Metabolism in Skin: Partial Purification and Properties of the Enzyme from Neonatal Rat Skin. *J. Biochem. Toxicol.* 1986, 1, 83–97.
18. Korkina, L.; De Luca, C.; Pastore, S. Plant Polyphenols and Human Skin: Friends or Foes. *Ann. N. Y. Acad. Sci.* 2012, 1259, 77–86.
19. Sirerol, J.A.; Feddi, F.; Mena, S.; Rodriguez, M.L.; Sirera, P.; Aupí, M.; Pérez, S.; Asensi, M.; Ortega, A.; Estrela, J.M. Topical Treatment with Pterostilbene, a Natural Phytoalexin, Effectively Protects Hairless Mice against UVB Radiation-Induced Skin Damage and Carcinogenesis. *Free Radic. Biol. Med.* 2015, 85, 1–11.
20. Ruiz, M.J.; Fernández, M.; Picó, Y.; Mañes, J.; Asensi, M.; Carda, C.; Asensio, G.; Estrela, J.M. Dietary Administration of High Doses of Pterostilbene and Quercetin to Mice Is Not Toxic. *J. Agric. Food Chem.* 2009, 57, 3180–3186.
21. Majeed, M.; Bani, S.; Natarajan, S.; Pandey, A.; Naveed, S. Evaluation of 90 Day Repeated Dose Oral Toxicity and Reproductive/Developmental Toxicity of 3'-Hydroxypterostilbene in Experimental Animals. *PLoS ONE* 2017, 12, e0172770.
22. Riche, D.M.; McEwen, C.L.; Riche, K.D.; Sherman, J.J.; Wofford, M.R.; Deschamp, D.; Griswold, M. Analysis of Safety from a Human Clinical Trial with Pterostilbene. *J. Toxicol.* 2013, 2013, 463595.
23. Priego, S.; Feddi, F.; Ferrer, P.; Mena, S.; Benlloch, M.; Ortega, A.; Carretero, J.; Obrador, E.; Asensi, M.; Estrela, J.M. Natural Polyphenols Facilitate Elimination of HT-29 Colorectal Cancer Xenografts by Chemoradiotherapy: A Bcl-2- and Superoxide Dismutase 2-Dependent Mechanism. *Mol. Cancer Ther.* 2008, 7, 3330–3342.
24. Estrela, J.M.; Mena, S.; Obrador, E.; Benlloch, M.; Castellano, G.; Salvador, R.; Dellinger, R.W. Polyphenolic Phytochemicals in Cancer Prevention and Therapy: Bioavailability versus Bioefficacy. *J. Med. Chem.* 2017, 60, 9413–9436.
25. Azzolini, M.; Mattarei, A.; La Spina, M.; Fanin, M.; Chiodarelli, G.; Romio, M.; Zoratti, M.; Paradisi, C.; Biasutto, L. New Natural Amino Acid-Bearing Prodrugs Boost Pterostilbene's Oral Pharmacokinetic and Distribution Profile. *Eur. J. Pharm. Biopharm.* 2017, 115, 149–158.
26. Hsieh, M.-T.; Huang, L.-J.; Wu, T.-S.; Lin, H.-Y.; Morris-Natschke, S.L.; Lee, K.-H.; Kuo, S.-C. Synthesis and Antitumor Activity of Bis(Hydroxymethyl)Propionate Analogs of Pterostilbene in Cisplatin-Resistant Human Oral Cancer Cells. *Bioorg. Med. Chem.* 2018, 26, 3909–3916.
27. Shaikh, J.; Ankola, D.D.; Beniwal, V.; Singh, D.; Kumar, M.N.V.R. Nanoparticle Encapsulation Improves Oral Bioavailability of Curcumin by at Least 9-Fold When Compared to Curcumin Administered with Piperine as Absorption Enhancer. *Eur. J. Pharm. Sci.* 2009, 37, 223–230.
28. Liu, Q.; Chen, J.; Qin, Y.; Jiang, B.; Zhang, T. Encapsulation of Pterostilbene in Nanoemulsions: Influence of Lipid Composition on Physical Stability, in Vitro Digestion, Bioaccessibility, and Caco-2 Cell Monolayer Permeability. *Food Funct.* 2019, 10, 6604–6614.
29. Bansal, S.S.; Goel, M.; Aqil, F.; Vadhanam, M.V.; Gupta, R.C. Advanced Drug Delivery Systems of Curcumin for Cancer Chemoprevention. *Cancer Prev. Res. Phila.* 2011, 4, 1158–1171.
30. Liu, K.-F.; Liu, Y.-X.; Dai, L.; Li, C.-X.; Wang, L.; Liu, J.; Lei, J.-D. A Novel Self-Assembled PH-Sensitive Targeted Nanoparticle Platform Based on Antibody-4arm-Polyethylene Glycol-Pterostilbene Conjugates for Co-Delivery of

31. Liu, Q.; Chen, J.; Qin, Y.; Jiang, B.; Zhang, T. Zein/Fucoidan-Based Composite Nanoparticles for the Encapsulation of Pterostilbene: Preparation, Characterization, Physicochemical Stability, and Formation Mechanism. *Int. J. Biol. Macromol.* 2020, 158, 461–470.
32. Romio, M.; Morgese, G.; Trachsel, L.; Babity, S.; Paradisi, C.; Brambilla, D.; Benetti, E.M. Poly(2-Oxazoline)-Pterostilbene Block Copolymer Nanoparticles for Dual-Anticancer Drug Delivery. *Biomacromolecules* 2018, 19, 103–111.
33. Coimbra, M.; Isacchi, B.; van Bloois, L.; Torano, J.S.; Ket, A.; Wu, X.; Broere, F.; Metselaar, J.M.; Rijcken, C.J.F.; Storm, G.; et al. Improving Solubility and Chemical Stability of Natural Compounds for Medicinal Use by Incorporation into Liposomes. *Int. J. Pharm.* 2011, 416, 433–442.
34. Liechty, W.B.; Peppas, N.A. Expert Opinion: Responsive Polymer Nanoparticles in Cancer Therapy. *Eur. J. Pharm. Biopharm.* 2012, 80, 241–246.
35. Mak, K.-K.; Wu, A.T.H.; Lee, W.-H.; Chang, T.-C.; Chiou, J.-F.; Wang, L.-S.; Wu, C.-H.; Huang, C.-Y.F.; Shieh, Y.-S.; Chao, T.-Y.; et al. Pterostilbene, a Bioactive Component of Blueberries, Suppresses the Generation of Breast Cancer Stem Cells within Tumor Microenvironment and Metastasis via Modulating NF-KB/MicroRNA 448 Circuit. *Mol. Nutr. Food Res.* 2013, 57, 1123–1134.
36. Schultheiss, N.; Bethune, S.; Henck, J.-O. Nutraceutical Cocrystals: Utilizing Pterostilbene as a Cocrystal Former. *CrystEngComm* 2010, 12, 2436–2442.
37. Bethune, S.J.; Schultheiss, N.; Henck, J.-O. Improving the Poor Aqueous Solubility of Nutraceutical Compound Pterostilbene through Cocrystal Formation. *Cryst. Growth Des.* 2011, 11, 2817–2823.
38. Bracht, J.W.P.; Karachaliou, N.; Berenguer, J.; Pedraz-Valdunciel, C.; Filipiska, M.; Codony-Servat, C.; Codony-Servat, J.; Rosell, R. Osimertinib and Pterostilbene in EGFR-Mutation-Positive Non-Small Cell Lung Cancer (NSCLC). *Int. J. Biol. Sci.* 2019, 15, 2607–2614.
39. Shin, H.J.; Han, J.M.; Choi, Y.S.; Jung, H.J. Pterostilbene Suppresses Both Cancer Cells and Cancer Stem-Like Cells in Cervical Cancer with Superior Bioavailability to Resveratrol. *Molecules* 2020, 25, 228.
40. Hsu, Y.-H.; Chen, S.-Y.; Wang, S.-Y.; Lin, J.-A.; Yen, G.-C. Pterostilbene Enhances Cytotoxicity and Chemosensitivity in Human Pancreatic Cancer Cells. *Biomolecules* 2020, 10, 709.
41. Wen, W.; Lowe, G.; Roberts, C.M.; Finlay, J.; Han, E.S.; Glackin, C.A.; Dellinger, T.H. Pterostilbene Suppresses Ovarian Cancer Growth via Induction of Apoptosis and Blockade of Cell Cycle Progression Involving Inhibition of the STAT3 Pathway. *Int. J. Mol. Sci.* 2018, 19, 1983.
42. Chang, H.-P.; Lu, C.-C.; Chiang, J.-H.; Tsai, F.-J.; Juan, Y.-N.; Tsao, J.-W.; Chiu, H.-Y.; Yang, J.-S. Pterostilbene Modulates the Suppression of Multidrug Resistance Protein 1 and Triggers Autophagic and Apoptotic Mechanisms in Cisplatin-Resistant Human Oral Cancer CAR Cells via AKT Signaling. *Int. J. Oncol.* 2018, 52, 1504–1514.
43. Elsherbini, A.M.; Sheweita, S.A.; Sultan, A.S. Pterostilbene as a Phytochemical Compound Induces Signaling Pathways Involved in the Apoptosis and Death of Mutant P53-Breast Cancer Cell Lines. *Nutr. Cancer* 2020, 1–9.
44. Mannal, P.; McDonald, D.; McFadden, D. Pterostilbene and Tamoxifen Show an Additive Effect against Breast Cancer in Vitro. *Am. J. Surg.* 2010, 200, 577–580.
45. Mori, S.; Kishi, S.; Honoki, K.; Fujiwara-Tani, R.; Moriguchi, T.; Sasaki, T.; Fujii, K.; Tsukamoto, S.; Fujii, H.; Kido, A.; et al. Anti-Stem Cell Property of Pterostilbene in Gastrointestinal Cancer Cells. *Int. J. Mol. Sci.* 2020, 21, 9347.
46. Lin, V.C.-H.; Tsai, Y.-C.; Lin, J.-N.; Fan, L.-L.; Pan, M.-H.; Ho, C.-T.; Wu, J.-Y.; Way, T.-D. Activation of AMPK by Pterostilbene Suppresses Lipogenesis and Cell-Cycle Progression in P53 Positive and Negative Human Prostate Cancer Cells. *J. Agric. Food Chem.* 2012, 60, 6399–6407.
47. Mannal, P.W.; Alosi, J.A.; Schneider, J.G.; McDonald, D.E.; McFadden, D.W. Pterostilbene Inhibits Pancreatic Cancer in Vitro. *J. Gastrointest. Surg.* 2010, 14, 873–879.
48. Mena, S.; Rodríguez, M.L.; Ponsoda, X.; Estrela, J.M.; Jäättelä, M.; Ortega, A.L. Pterostilbene-Induced Tumor Cytotoxicity: A Lysosomal Membrane Permeabilization-Dependent Mechanism. *PLoS ONE* 2012, 7, e44524.
49. Chatterjee, K.; Mukherjee, S.; Vanmanan, J.; Banerjee, P.; Fata, J.E. Dietary Polyphenols, Resveratrol and Pterostilbene Exhibit Antitumor Activity on an HPV E6-Positive Cervical Cancer Model: An in Vitro and in Vivo Analysis. *Front. Oncol.* 2019, 9, 352.
50. Tam, K.-W.; Ho, C.-T.; Tu, S.-H.; Lee, W.-J.; Huang, C.-S.; Chen, C.-S.; Wu, C.-H.; Lee, C.-H.; Ho, Y.-S. α -Tocopherol Succinate Enhances Pterostilbene Anti-Tumor Activity in Human Breast Cancer Cells in Vivo and in Vitro. *Oncotarget* 2018, 9, 4593–4606.

51. Pan, C.; Hu, Y.; Li, J.; Wang, Z.; Huang, J.; Zhang, S.; Ding, L. Estrogen Receptor-A36 Is Involved in Pterostilbene-Induced Apoptosis and Anti-Proliferation in in Vitro and in Vivo Breast Cancer. *PLoS ONE* 2014, 9, e104459.
52. Dhar, S.; Kumar, A.; Rimando, A.M.; Zhang, X.; Levenson, A.S. Resveratrol and Pterostilbene Epigenetically Restore PTEN Expression by Targeting OncomiRs of the MiR-17 Family in Prostate Cancer. *Oncotarget* 2015, 6, 27214–27226.
53. Su, C.-M.; Lee, W.-H.; Wu, A.T.H.; Lin, Y.-K.; Wang, L.-S.; Wu, C.-H.; Yeh, C.-T. Pterostilbene Inhibits Triple-Negative Breast Cancer Metastasis via Inducing MicroRNA-205 Expression and Negatively Modulates Epithelial-to-Mesenchymal Transition. *J. Nutr. Biochem.* 2015, 26, 675–685.
54. McCormack, D.E.; Mannal, P.; McDonald, D.; Tighe, S.; Hanson, J.; McFadden, D. Genomic Analysis of Pterostilbene Predicts Its Antiproliferative Effects against Pancreatic Cancer in Vitro and in Vivo. *J. Gastrointest. Surg.* 2012, 16, 1136–1143.
55. Kong, Y.; Chen, G.; Xu, Z.; Yang, G.; Li, B.; Wu, X.; Xiao, W.; Xie, B.; Hu, L.; Sun, X.; et al. Pterostilbene Induces Apoptosis and Cell Cycle Arrest in Diffuse Large B-Cell Lymphoma Cells. *Sci. Rep.* 2016, 6, 37417.
56. Wakimoto, R.; Ono, M.; Takeshima, M.; Higuchi, T.; Nakano, S. Differential Anticancer Activity of Pterostilbene against Three Subtypes of Human Breast Cancer Cells. *Anticancer Res.* 2017, 37, 6153–6159.
57. Li, K.; Dias, S.J.; Rimando, A.M.; Dhar, S.; Mizuno, C.S.; Penman, A.D.; Lewin, J.R.; Levenson, A.S. Pterostilbene Acts through Metastasis-Associated Protein 1 to Inhibit Tumor Growth, Progression and Metastasis in Prostate Cancer. *PLoS ONE* 2013, 8, e57542.
58. Wen, W.; Lowe, G.; Roberts, C.M.; Finlay, J.; Han, E.S.; Glackin, C.A.; Dellinger, T.H. Pterostilbene, a Natural Phenolic Compound, Synergizes the Antineoplastic Effects of Megestrol Acetate in Endometrial Cancer. *Sci. Rep.* 2017, 7, 12754.
59. Wang, D.; Guo, H.; Yang, H.; Wang, D.; Gao, P.; Wei, W. Pterostilbene, An Active Constituent of Blueberries, Suppresses Proliferation Potential of Human Cholangiocarcinoma via Enhancing the Autophagic Flux. *Front. Pharmacol.* 2019, 10, 1238.
60. Xie, B.; Xu, Z.; Hu, L.; Chen, G.; Wei, R.; Yang, G.; Li, B.; Chang, G.; Sun, X.; Wu, H.; et al. Pterostilbene Inhibits Human Multiple Myeloma Cells via ERK1/2 and JNK Pathway In Vitro and In Vivo. *Int. J. Mol. Sci.* 2016, 17, 1927.
61. Paul, S.; DeCastro, A.J.; Lee, H.J.; Smolarek, A.K.; So, J.Y.; Simi, B.; Wang, C.X.; Zhou, R.; Rimando, A.M.; Suh, N. Dietary Intake of Pterostilbene, a Constituent of Blueberries, Inhibits the Beta-Catenin/P65 Downstream Signaling Pathway and Colon Carcinogenesis in Rats. *Carcinogenesis* 2010, 31, 1272–1278.
62. Guo, L.; Tan, K.; Wang, H.; Zhang, X. Pterostilbene Inhibits Hepatocellular Carcinoma through P53/SOD2/ROS-Mediated Mitochondrial Apoptosis. *Oncol. Rep.* 2016, 36, 3233–3240.
63. Tsai, M.-L.; Lai, C.-S.; Chang, Y.-H.; Chen, W.-J.; Ho, C.-T.; Pan, M.-H. Pterostilbene, a Natural Analogue of Resveratrol, Potently Inhibits 7,12-Dimethylbenz[a]Anthracene (DMBA)/12-O-Tetradecanoylphorbol-13-Acetate (TPA)-Induced Mouse Skin Carcinogenesis. *Food Funct.* 2012, 3, 1185–1194.
64. Huynh, T.-T.; Lin, C.-M.; Lee, W.-H.; Wu, A.T.H.; Lin, Y.-K.; Lin, Y.-F.; Yeh, C.-T.; Wang, L.-S. Pterostilbene Suppressed Irradiation-Resistant Glioma Stem Cells by Modulating GRP78/MiR-205 Axis. *J. Nutr. Biochem.* 2015, 26, 466–475.
65. Chiou, Y.-S.; Tsai, M.-L.; Nagabhushanam, K.; Wang, Y.-J.; Wu, C.-H.; Ho, C.-T.; Pan, M.-H. Pterostilbene Is More Potent than Resveratrol in Preventing Azoxymethane (AOM)-Induced Colon Tumorigenesis via Activation of the NF-E2-Related Factor 2 (Nrf2)-Mediated Antioxidant Signaling Pathway. *J. Agric. Food Chem.* 2011, 59, 2725–2733.