Potential of grape polyphenols as breast cancer therapeutics

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Abstract
Introduction
Grape and red wine polyphenols have long been purported to have multiple health benefits. Although convincing clinical data are still lacking, recent experimental studies have demonstrated the utility of grape polyphenols as anticancer compounds. This review discusses the potential of the major polyphenols from grape and red wine, resveratrol, quercetin and catechin, as alternative therapeutics for breast cancer.

Discussion
Accumulated data from in vitro studies with breast cancer cell lines and in vivo studies with rodent models demonstrate a pro-apoptotic, anti-proliferative, anti-invasive, anti-angiogenic and anti-metastatic role for grape polyphenols in breast cancer. At the molecular level, the anticancer effects of grape polyphenols have been attributed to the inhibition of a number of cancer-promoting pathways that include oestrogen receptor, growth factor receptor, mitogen-activated protein kinases, phosphoinositol 3-kinase/Akt/mammalian target of rapamycin pathway.

Conclusion
Appropriate pre-clinical and clinical studies to determine the efficacy, correct dosage and combination of grape polyphenols are warranted before the establishment of guidelines for the treatment of breast cancer patients, those at high-risk and survivors.

Review

Introduction
The health benefits of plant-derived polyphenols in our diet are becoming more evident and better understood. Plant polyphenols are structurally diverse micronutrients that consist mostly of flavonoids (abundant in our diet), phenolic acids, stilbenes and lignans. Grape polyphenols, enriched in grape skin and grape seeds, and thus in red wine, exert a myriad of beneficial effects that include: antioxidant, anti-carcinogenic, anti-inflammatory, neuroprotective, cardioprotective, anti-allergic, anti-diarrhoeal, anti-ulcer, antibiotic, anti-ageing, anti-angiogenic, vasorelaxing and anti-thrombotic properties.

A number of studies have implicated grape polyphenols in cancer prevention and inhibition of cancer progression due to their antioxidant and pro-apoptotic effects as well as the inhibition of pro-cancer molecular pathways. Moreover, these compounds are structurally similar to the female hormone oestrogen and can act as selective oestrogen receptor (ER) modulators and induce differential gene expression via ERα and ERβ. Thus, these phytoestrogens are especially relevant for gynaecological cancers, such as breast cancer. Additionally, recent research has implicated plant polyphenols as safe alternatives to sensitising tumours to chemotherapeutics or radiation via inhibition of resistant pathways. However, although a definitive role for grape polyphenols as cancer preventives has been demonstrated, the formulations, concentrations and the type and stage of cancers that will benefit from grape polyphenols remain to be clarified. This review discusses the potential of grape polyphenols as breast cancer therapeutics.

Discussion
The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

Grape polyphenols as breast cancer preventives and alternative therapeutics
Breast cancer is the most commonly diagnosed form of cancer, and the second cause of cancer deaths among women, with 232,340 new cases estimated for 2013. Consequently, it is critical to find new alternatives for breast cancer prevention and cure. Of these, grape polyphenols are an attractive alternative. However, even though epidemiological studies have associated grape consumption with reduced breast cancer risk,
consumption of grape polyphenols from 'over-the-counter' nutraceutical preparations still poses a potential health hazard. This is because the effects of individual or combined grape polyphenols, and their effective concentrations and combinations for breast cancer prevention are yet to be elucidated.

The anticancer role of grape polyphenols has been demonstrated using numerous in vitro and in vivo systems, including breast cancer models. Additionally, grape-derived polyphenols, grape seed extract (GSE) and grape juice constituents have been shown to inhibit breast cancer initiation and reduce cancer in rodent models7–9,22–26. GSE was also shown to block the activity of Akt in breast cancer cells, an important signalling intermediate that regulates cell survival, protein synthesis, proliferation and invasion27. The cellular mechanisms of grape polyphenol-mediated inhibition of breast cancer cell proliferation have been attributed to: (1) binding to ER and blocking ER-mediated gene transcription, (2) interaction with membrane ER, to regulate several protein kinases and transcription factors, (3) inhibition of growth factor receptor signalling, (4) acting as aromatase inhibitors that prevent the conversion of androgens to oestrogen, and (5) induction of apoptosis5,28.

Approximately 70% of the red wine polyphenols consist of 1% resveratrol (stilbene), 6% quercetin (flavone) and 65%–70% catechin (flavan-3-ol)5,9,29. Due to their structural similarity to oestrogen (Figure 1), grape polyphenols can exert oestrogenic or anti-oestrogenic effects in breast cancer14,30. However, our group and others have shown that grape polyphenols also inhibit ERα negative (−) breast cancer progression and metastasis5,18,31–33. Therefore, grape polyphenols also regulate ER-independent signalling pathways.

**Resveratrol**

Resveratrol acts as a cancer preventive due to its antioxidant, pro-apoptotic, anti-proliferative, anti-inflammatory, anti-angiogenic and anti-invasive properties34,35. Since the initial report of resveratrol acting as a cancer preventive in a skin cancer model36, a great deal of attention has been focused on the anticancer effects of resveratrol. However, a complete understanding of the myriad of signalling pathways regulated by resveratrol, the effects of resveratrol on different types of cancers and the relevant doses still remain elusive37.

Resveratrol binds both ERα and ERβ with comparable affinity, but with 7000-fold lower affinity than oestrogen38,39. Moreover, resveratrol can modulate cancer via alternative pathways. The most widely studied mediators of the anticancer effects of resveratrol are mitogen-activated protein kinases (MAPKs), nuclear factor kB (NFκB) and phosphoinositide 3-kinase (PI3-K)/Akt/mammalian target of rapamycin (mTOR) pathways that regulate cancer cell proliferation, survival and migration/invasion, as well as immune responses and inflammation34,40–44 (Figure 2). Resveratrol-mediated inhibition of the PI3-K/Akt/mTOR pathway, which plays a central role in the regulation of protein synthesis, cell proliferation, tumour growth and metastasis, and chemotherapy resistance, is thought to be relevant for the anticancer functions of resveratrol45.

In breast cancer, resveratrol has been implicated in the inhibition of multistage carcinogenesis in vitro46. Resveratrol promotes breast cancer cell apoptosis by regulating extracellular regulated kinase, c-Jun N-terminal kinase, p38 MAPKs47–49 and signal transducer and activator of transcription 340. Resveratrol also protects breast cells from carcinogenic oestrogen metabolites50, impedes the non-genomic induction of oestrogen

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**Figure 1:** Structures of resveratrol, quercetin and catechin. Structures of the stilbene resveratrol, flavonol quercetin and flavan-3-ol catechin are compared with oestrogen. These polyphenols can act as antioxidants and may have oestrogenic/anti-oestrogenic activity due to similarity in distance between the terminal hydroxyl groups of oestrogen that can interact with oestrogen receptors.

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**FOR CITATION PURPOSES:** Castillo-Pichardo L, Rivera-Rivera A, Dharmawardhane S. Potential of grape polyphenols as breast cancer therapeutics. OA Alternative Medicine 2013 Apr 01;1(1):9.

Competing interests: none declared. Conflict of interests: none declared. All authors contributed to conception and design of the study, acquisition of data, analysis and interpretation of data, and all authors read and approved the final manuscript. All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.
Figure 2: Effects of individual and combined resveratrol, quercetin and catechin on cancer cell signalling. The discussed effects of individual and combined grape polyphenols on tumourigenic signalling pathways are presented in this simplified signalling scheme. Resveratrol (R) and quercetin (Q) have been shown to bind to ER, including the subpopulation of membrane ER to affect non-genomic signalling via crosstalk with EGFR, and either activate or inhibit EGFR signalling in a concentration-dependent manner. EGFR signalling activates a number of cancer-promoting pathways. These include the following signalling sequelae (from left to right): (1) Activation of phospholipase C (PLC) to generate protein kinase C (PKC) and calcium signalling is inhibited by combined red wine polyphenols (RWP). (2) The janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway that regulates growth, survival and differentiation is inhibited by resveratrol. (3) Both resveratrol and quercetin are direct inhibitors of phosphoinositide 3-kinase (PI3-K), an enzyme that activates the pro-survival oncoprotein Akt. Akt activity, is inhibited by low concentrations of combined resveratrol, quercetin and catechin (1:1:1) (RQC). The Akt downstream effector NFκB that regulates cancer cell survival and inflammation is inhibited by resveratrol, RQC and RWP. (4) Growth factor receptor signalling (as well as nutrients, energy, stress and hormone receptors) activate the mTOR kinase complex mTORC1, which includes mTOR kinase and the regulatory-associated protein of mTOR (Raptor). Activated Akt regulates mTORC1 by an inhibitory phosphorylation of tuberous sclerosis complex 2 (TSC2), a GTPase-activating protein for Rheb, which is the GTP-binding protein that activates mTOR. Activated mTORC1 phosphorylates eukaryotic initiation factor 4E binding protein (4EBP) and p70S6 ribosomal kinase (p70S6K) to regulate cell growth by initiating protein synthesis and ribosome biogenesis. RQC was shown to inhibit p70S6K activity. RQC also activates an important negative regulator of mTOR signalling, AMP-activated protein kinase (AMPK). AMPK inhibits mTORC1 by activating TSC2 and by direct inhibition of Raptor. (5) The Ras/mitogen-activated protein kinase pathway, an important regulator of cell growth, is inhibited by individual resveratrol and quercetin. (6) Individual resveratrol has concentration-dependent effects on focal adhesion kinase (FAK) and the small GTPases Rac and Cdc42 that regulate motility and metastasis.
signalling and inhibits ERα expression in breast cancer cells.

However, much of the data on the anticancer properties of resveratrol have used high (up to 100 μM) concentrations that are rarely achieved via dietary consumption of grape or grape products. In vivo animal studies that demonstrated a breast cancer preventive role for resveratrol used 25–200 mg/kg resveratrol and reported reduced mammary tumour growth. Intriguingly, our studies have shown a biphasic concentration-dependent action for resveratrol in breast cancer cells. Resveratrol at low concentrations (≤5 μM) promoted and at high concentrations (>5 μM) inhibited pro-migratory actin structures and focal adhesions, and the related activities of focal adhesion kinase, Akt, and the small GTPase Rac. We have corroborated these in vitro studies in vivo, where we recently determined the effect of 0.5, 5 or 50 mg/kg body weight (BW) of dietary resveratrol on breast cancer progression in athymic nude or severe combined immunodeficiency (SCID) mice with mammary fatpad tumours from ER(−) MDA-MB-435 cells or ERα(−) ERβ(+) MDA-MB-231 cells, respectively. We found that resveratrol, at all concentrations tested, resulted in a dramatic increase in tumour growth and metastasis, with a parallel increase in tumoural Rac activity. However, others have reported reduced tumour growth of MDA-MB-231 in nude mice at 25 mg/kg and prolonged onset of tumours and reduced metastases in a HER2/neu spontaneous breast cancer model following 4 μg resveratrol/mouse, as well as reduced metastasis in a mouse model following 100 and 200 mg/kg BW resveratrol. Resveratrol has also been shown to reduce mammary tumour growth in rats by some groups, while others have reported increased mammary tumour growth in rats in response to 100 mg/kg resveratrol. These discrepancies may be due to differences in rodent models, the concentration or route of resveratrol administration. Overall, the published data indicate that, even though resveratrol acts as a cancer preventive at high concentrations, caution must be exercised with resveratrol treatment for breast cancer patients or survivors.

Clinical trials on resveratrol have mainly focused on pharmacokinetics and metabolism, and the effect of resveratrol in colorectal cancer, where high doses of resveratrol were shown to be effective. More studies need to be performed to further investigate the optimal dose, long-term effects and molecular mechanisms of resveratrol before large-scale clinical trials on the anticancer effects of resveratrol can be conducted.

**Quercetin**

Quercetin, a flavonoid with potent antioxidant and anti-proliferative effects, comprises about 6% of total polyphenols in red wine. Quercetin also binds ERs and ERβ to induce ER-mediated gene expression and preferentially signals through ERβ. Additionally, quercetin decreases mammmary tumour growth and breast cancer cell viability, and induces cell cycle arrest and apoptosis. Quercetin has also been shown to have anti-migration/invasion properties and inhibit survival signalling and tumour growth in rodents. A number of pro-cancer cell signalling pathways have been identified as targets of quercetin at high, pharmacological concentrations. Some of these effects may be due to direct inhibition of PI3-K, because quercetin was the lead compound in the design of the commercial PI3-K inhibitor VX294002. We recently reported that quercetin, at low concentrations, inhibits mTOR activity of breast cancer cells, both via inhibition of Akt and by activation of the mTOR negative regulator adenosine monophosphate (AMP) kinase (AMPK); thus, implicating quercetin in the inhibition of mTOR activity via a dual mechanism (Figure 2). A recent study also demonstrated that quercetin potentiates the cancer therapeutic doxorubicin, in ER(+) breast cancer cells. However, a better understanding of the anti-cancer effects and molecular mechanisms of quercetin at a broad range of concentrations is necessary before clinical trials on the anti-tumour effects of quercetin can be conducted.

**Catechin**

Most studies on the cancer preventive properties of catechins have used catechins from green tea, which is comprised of a mixture of epigallocatechin gallate, epigallocatechin and epicatechin gallate. However, not much is known about the anticancer properties of the monomeric non-gallated catechin, the major grape polyphenol. Monomeric catechin was found to delay tumour onset in a spontaneous mouse model. Additionally, a hydrated form of catechin was reported to induce apoptosis in breast cancer cells by increased expression of p53 and caspases. Therefore, more studies are needed on the efficacy of monomeric catechin as a breast cancer preventive.

**Combined resveratrol, quercetin and catechin**

Much of the data on the cancer preventive effects of grape polyphenols has been generated from ER(+) tissue culture cell lines treated with high concentrations of individual polyphenols. As discussed earlier, these studies have demonstrated the cancer-preventive efficacy of individual treatments of resveratrol, quercetin or catechin at pharmacological concentrations. Our group has studied the effects of individual and combined resveratrol, quercetin and catechin (RQC), at low physiologically relevant concentrations on cellular processes important for breast cancer progression, using metastatic ER(−) breast cancer models. We demonstrated that an
equimolar combination of RQC, at low concentrations (0.5–5 µM each), is more effective than the individual compounds in inhibiting metastatic breast cancer cell viability, cell cycle progression, cell migration and the tumourigenic PI3K/Akt/mTOR pathway (Table 1, Figure 2). Moreover, the RQC formulation was effective at inducing apoptosis and regulating apoptotic signalling proteins. We also demonstrated that RQC (0.5, 5 or 25 mg/kg BW each) can suppress growth and metastasis of MDA-MB-231 and MDA-MB-435 mammary fatpad xenografts in nude mice (Table 1). At 5 mg/kg BW each, RQC specifically inhibited bone and liver metastasis of the MDA-MB-435 bone metastatic variant. These data suggest that, although this formulation may not suppress intravasation and shedding of metastatic cancer cells into the vasculature, it can specifically inhibit the colonisation of metastatic cancer cells in the bone and liver.

The potential of grape polyphenols to reduce mammary tumour growth has also been demonstrated with GSE, which reduced MDA-MB-231 xenografts via decreased angiogenesis. Moreover, grape polyphenols, isolated from red wine polyphenols (RWPs), dramatically inhibited the growth of MDA-MB-231 xenografts and reduced NfκB, phospholipase C and calcium signalling pathways. Grape polyphenols were also implicated in NfκB inhibition by our studies, where dietary RQC increased the expression of the inhibitor of NfκB (NFKBIA or IkBα) in mammary tumours (Figure 2).

We recently demonstrated an RQC-mediated dual inhibition of the mTOR pathway, mTOR is a pivotal signal integrator for nutrients, growth factors, hormones, stress and energy. The mTOR pathway regulates cancer progression by promoting cell growth and therapy resistance. RQC inhibited mTOR activity, both via inhibition of Akt and activation of AMPK, a stress-sensing enzyme that requires AMP for activation and regulates cell growth, autophagy and metabolism (Figure 2). Therefore, these studies implicate the RQC formulation in the inhibition of breast cancer growth, metastasis and therapy resistance.

The potential of RQC to sensitize breast cancers to the epidermal growth factor receptor (EGFR) therapeutic gefitinib was demonstrated by using the gefitinib-resistant MDA-MB-231 metastatic breast cancer cell line. We recently reported that RQC inhibits mTOR signalling even in the presence of gefitinib and that combined RQC and gefitinib is more effective than individual RQC or gefitinib at inhibiting cell proliferation and reducing mammary tumour growth and metastasis in SCID mice (Figure 3). Current advances in breast cancer therapy include dual therapeutic strategies with mTOR inhibitors to overcome resistance to anti-EGFR/HER2 and anti-oestrogen therapeutics. However, such combination therapy with chemical inhibitors often results in side effects and failure in clinical trials due to cytotoxicity and further development of resistance. A safer alternative is the use of common dietary compounds with low toxicity that can inhibit therapy resistance pathways. This is supported by recent studies that have elucidated a chemosensitising role for grape polyphenols, such as the RQC formulation, as well as grape seed and skin extracts. Further clinical studies are warranted to investigate the efficacy of grape polyphenols as safe, non-toxic and economically feasible alternatives in combination therapy for aggressive breast cancers.

Bioavailability of grape polyphenols

To establish polyphenols as dietary and therapeutic anticancer compounds, it is important to first understand their processing and bioavailability in humans. RQCs are present in red wine at an average of 2.7 mg/L (0.1–14.3 mg/L) resveratrol, 8.3 mg/L (up to 13.9–14.5 mg/L) quercetin and 68.1 mg/L catechin (up to 270 mg/L) in plasma and urine of rodents and humans. However, grape polyphenols are detectable in plasma and urine of rodents and humans.

Dietary polyphenols are usually consumed in the form of esters.
Combined resveratrol, quercetin and catechin (RQC) in therapy resistance. RQC inhibits mTOR activity via inhibition of Akt and activation of AMPK. RQC-mediated inhibition of Akt/mTOR signalling that is elevated during therapy resistance, leads to chemosensitisation of anti-EGFR therapy in therapy resistant breast cancer cells.

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Therefore, following consumption, the aglycones are found in the serum at low concentrations, with the majority as glucuronide and sulphate conjugates. Such extensive conjugation of polyphenols is thought to be one of the main reasons for the low oral bioavailability of dietary flavonoids and other polyphenols. Free (aglycone) polyphenols in the serum of humans following consumption was shown to be 1.7%–1.9% resveratrol, 17.2%–26.9% quercetin, and 1.1%–6.5% catechin, while more than 80% of these compounds were absorbed. However, a different study reported peak concentrations of resveratrol in humans to be <10 ng/mL at 0.5–2 h after oral consumption. The different methods of detection and absorption of aglycone forms of polyphenols in tissue may account for the variability and low amounts of polyphenols detected from serum/plasma and urine. Quercetin, for instance, is generally undetected in plasma; however, it is present in considerable amounts in tissues. This is probably because quercetin is more lipophilic than its glucuronosyl- and sulpho-conjugated metabolites; consequently quercetin may remain in the tissue interacting with cell membrane phospholipids. This ability of polyphenolics to penetrate tissues may explain their potent anticancer effects even when low levels are detected in urine and serum.

**Clinical trials of grape polyphenols as anti-breast cancer compounds**

Surprisingly, comprehensive studies on the efficacy of grape polyphenols for breast cancer patients have yet to be conducted. A search of the National Institutes of Health ‘ClinicalTrials.gov’ website for ‘grape polyphenols’, ‘red wine’ or ‘resveratrol’ and ‘breast cancer’, yielded only one trial on the efficacy of red wine versus white wine for breast cancer risk. Although a previous study reported that there was no difference between red or white wine consumption and breast cancer risk, this recent clinical trial determined that in postmenopausal women, moderate red wine consumption, and not white wine, reduced breast cancer risk, due to the efficacy of grape polyphenols as aromatase inhibitors. Therefore, more preclinical and clinical trials are needed for a comprehensive evaluation of the potential of grape polyphenols as anti-breast cancer compounds.

**Conclusion**

Grape polyphenols have immense potential as cancer therapeutics because they have demonstrated high efficacy against many types of cancer, including breast cancer. They are also absorbed and metabolised by humans, can be consumed orally, are inexpensive and readily available. In addition to their cancer preventive effects, grape polyphenols may also be useful as anti-breast cancer therapeutics, especially in combination with other cancer therapeutics, as non-toxic safe alternatives to potentiate current breast cancer therapies. However, to establish grape polyphenols as viable breast cancer therapeutics, more comprehensive pre-clinical and clinical trials that demonstrate the efficacy, optimal dose, effective combination and targeted type and stage of cancer are urgently needed.

**Abbreviations list**

- AMPK, AMP-activated protein kinase
- BW, body weight
- EGFR, epidermal growth factor receptor
- ER, oestrogen receptor
- FAK, focal adhesion kinase
- GSE, grape seed extract
- JAK, janus kinase
- MAPK, mitogen-activated protein kinase
- mTOR, mammalian target of rapamycin
- NFκB, nuclear factor κB
- PLC, phospholipase C
- Raptor, regulatory-associated protein of mTOR
- RQC, resveratrol, quercetin and catechin
- RWP, red wine polyphenol
- SCID, severe combined immunodeficiency
- STAT, signal transducer and activator of transcription
- TSC2, tuberous sclerosis complex 2
- 4EBP, 4E binding protein
- 4E-BP1, eukaryotic translation initiation factor 4E binding protein 1
- 4E-BP2, eukaryotic translation initiation factor 4E binding protein 2
- FAK, focal adhesion kinase
- EGFR, epidermal growth factor receptor
- ER, oestrogen receptor
- PLC, phospholipase C
- mTOR, mammalian target of rapamycin
- NFκB, nuclear factor κB
- p70S6K, p70S6 ribosomal kinase
- p53, tumour suppressor gene
- TSC2, tuberous sclerosis complex 2
- 4EBP, 4E binding protein
- 4E-BP1, eukaryotic translation initiation factor 4E binding protein 1
- 4E-BP2, eukaryotic translation initiation factor 4E binding protein 2
- FAK, focal adhesion kinase
- EGFR, epidermal growth factor receptor
- ER, oestrogen receptor
- PLC, phospholipase C
- mTOR, mammalian target of rapamycin
- NFκB, nuclear factor κB
- p70S6K, p70S6 ribosomal kinase
- PI3-K, phosphoinositol 3-kinase
- PKC, protein kinase C
- PLC, phospholipase C
- Raptor, regulatory-associated protein of mTOR
- RQC, resveratrol, quercetin and catechin
- RWP, red wine polyphenol
- SCID, severe combined immunodeficiency
- STAT, signal transducer and activator of transcription
- TSC2, tuberous sclerosis complex 2
- 4EBP, 4E binding protein

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