J Bioenerg Biomembr (2007) 39:59–63
DOI 10.1007/s10863-006-9053-y

MINIREVIEW

Resveratrol in cell fate decisions

Andrea Lisa Holme and Shazib Pervaiz

Published online: 17 February 2007
© Springer Science+Business Media, LLC 2007

Abstract Resveratrol, a polyphenolic phytoalexin, is one of the most extensively studied natural products, with wide ranging biological activity and tremendous clinical potential. First identified from fruits and plants, in particular grapes and wines, its positive effects on a variety of disease states have been unraveled over the past decade or so. Most noticeable are its anti-thrombogenic, anti-inflammatory, cardio-protective, neuro-protective, anti-aging, and cancer preventive and therapeutic activities. Recent data also indicate that depending upon the concentration/dose, resveratrol can trigger or block cell death signaling in tumor cells. Considering the heightened interest in this compound, here we present a short review on the biological activity of this remarkable compound, with a specific focus on its effects on cell survival and death signals.

Keywords Resveratrol · Mitochondria · Apoptosis · ROS

Introduction

Recent data suggest that depending upon its concentration and the cell type, resveratrol (RSV) could promote cell survival signaling or activate cell death pathways. These diverse effects, though seemingly contradictory in some settings, have sparked tremendous interest in studies aimed at understanding the biological effects of RSV for its potential clinical usefulness. Many of the cancer-related effects of RSV have been shown to be a function of cell cycle arrest or/and apoptosis induction. These effects are related to its ability to modulate the activity of key mediators of the cell cycle and cell survival. Here we present a short focused review on the effect of RSV on cell growth and carcinogenesis from the standpoint of the mitochondria and bioenergetics.

A. L. Holme
ROS Biology and Apoptosis Group, National University Medical Institutes, National University of Singapore, Singapore 117597

S. Pervaiz (✉)
Department of Physiology, Yong Loo Lin School of Medicine, and NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, 2 Medical Drive, MD9 #03-06, Singapore 117597
e-mail: phssp@nus.edu.sg

Resveratrol and its impact on human health

A plethora of naturally occurring compounds is under investigation for their chemopreventive and/or chemotherapeutic potential. Among these compounds is a family of polymers (viniferins) with strong anti-fungal properties which includes plant antibiotics known as phytoalexins (Soleas et al., 1997). A biological potent member of this group is resveratrol (RSV), a major active ingredient of stilbene phytoalexins first purified from the roots of the oriental medicinal plant *Polygonum Capsidatum* (Ko-jo-kon in Japanese) (Nonomura et al., 1963). The observations that RSV was one of the major active ingredients of a folk plant, known for its remedial effects against a host of disease states (Nonomura et al., 1963; Kubo et al., 1981), and that it was synthesized in response to fungal infection of grapevines (*Vitis vinifera*) (Langcake and Pryce, 1976) provided the early stimulus for unraveling the biological properties of this remarkable compound. Since the first reported detection of *trans*-RSV in grapevines in 1976 (Langcake and Pryce, 1976) and later in wine in 1992 (Siemann and Creasy, 1992), most of the work had focused on RSV in grapevines. This was mainly due to the fact that compounds found in grapevines were implicated in epidemiological data demonstrating an inverse correlation between red wine consumption and the incidence of cardiovascular disease – the "*French paradox*" (Kopp, 1998). Most

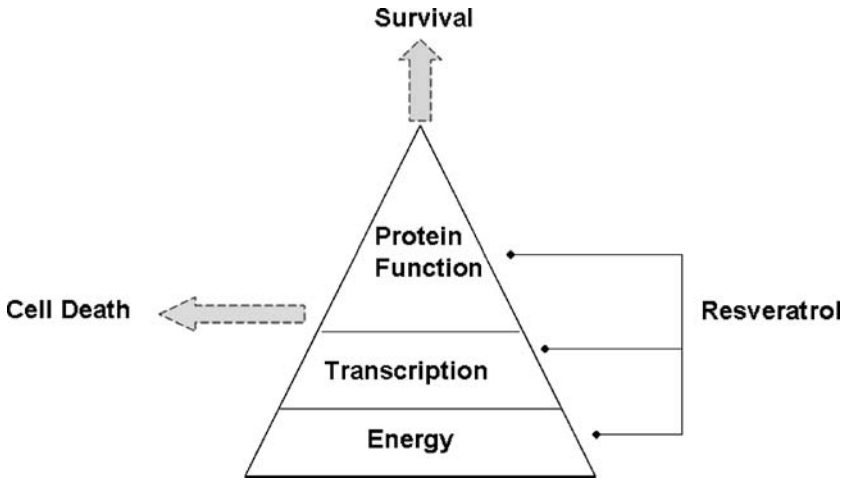

Fig. 1 Overview of the macro-cellular events that RSV targets, leading to cell death or survival. The outcome of which is dependent upon the concentration of RSV given and the genetic phenotype

of the earlier work with RSV was centered around its cardio-protective and anti-inflammatory effects and the mechanisms implicated include: anti-oxidant and anti-coagulant properties, association with lipoproteins, inhibition of low-density lipoprotein (LDL) oxidation, inhibition of platelet aggregation and polymorphonuclear cell activation, and induced vaso-relaxation [reviewed in (Pervaiz, 2003)]. These observations have been substantiated by recent data linking RSV to impaired migration and tube formation in endothelial cells and inhibition of the expression of adhesion molecules, thereby reducing thrombogenic potential (Baur and Sinclair, 2006). The effects of RSV on some important physiological pathways have been proposed as possible mechanisms for its observed cancer chemopreventive, cardio-protective, and neuroprotective activities as summarized in Fig. 1. Furthermore, it is structurally similar to the estrogenic agent diethylstilbestrol which confers upon it a pronounced *in vivo* estrogenic activity.

The real impetus to the interest in the biology of RSV was the report linking RSV to inhibition of tumor *initiation, promotion and progression* in a murine model (Jang et al., 1997). This was followed by observations implicating the apoptotic activity of RSV in its cancer preventive effect (Clement et al., 1998). Since then, a plethora of studies have unraveled intracellular circuits and molecular pathways directly or indirectly involved in RSV-mediated signaling (Aggarwal et al., 2004; Pervaiz, 2003). These include the involvement of both the death receptor pathway as well as the mitochondrial intrinsic pathway. In terms of the former, we demonstrated the involvement of the CD95-CD95L system in RSV-induced apoptosis of human leukemia and breast carcinoma cells, but not in activated peripheral blood mononuclear cells (Clement et al., 1998). These findings were further corroborated by two independent studies (Su et al., 2005; Wang et al., 2005). One possible scenario implicating death receptor signaling in the death inducing activity of RSV was the redistribution of CD95 (Apo1/Fas) and other death receptors of the TNF superfamily, such as TRAIL-R (TNF-related apoptosis inducing ligand receptor), to lipid rafts. Although, this redistribution of death receptors was insufficient to trigger cell death by its own, it had the effect of significantly amplifying death signaling upon exposure to death receptor agonists such as anti-CD95 or TRAIL (Fulda and Debatin, 2005) (Velthuis et al., 2002). Of note, lipid membrane structures, such as cholesterol – and/or sphingolipid-rich moieties of the plasma membrane (lipid rafts), actively participate in metabolic and signal transduction processes, particularly the ligation of CD95 death receptor (Cahuzac et al., 2006; Henkler et al., 2005; Dimanche-Boitrel et al., 2005).

It should be pointed out that the IC_{50} of RSV for proliferation inhibition was significantly lower for cancer cells (34 μM for leukemia cells) as compared to normal hematopoietic cells (59 μM) (Gautam et al., 2000). Similarly, a bolus treatment with RSV (80 μM for 20h) had a significantly stronger inhibitory effect on the colony forming ability of leukemia cells than the hematopoietic progenitors cells. Further corroborating the relatively higher sensitivity of cancer cells to RSV treatment, SV40-transformed human fibroblasts (WI38VA) were shown to be more sensitive to RSV than normal fibroblasts in modulating the expressions of pro- versus anti-apoptotic genes (Gosslau et al., 2005; Lu et al., 2001).

Calories, life span and resveratrol – the "French paradox"

The "*French paradox*" was the driving force behind the initial interest in the biological properties of the phytoalexin RSV; relatively lower rates of heart disease and cancer in populations from wine drinking nations, despite a diet high in saturated fat and cholesterol and/or a high blood cholesterol level. Polyunsaturated fatty acids are esterified

in membrane phospholipids and triglycerides in mammalian tissues, and mainly function to maintain membrane fluidity and substrate storage. Oxidative metabolism of these fatty acids to eicosanoids (collective name for prostaglandins, hydroxy-fatty acids and leukotrienes) depends on the availability of free non-esterified fatty acids. These fatty acids are substrates for three distinctively different enzymatic pathways; cyclooxygenase (COX), lipoxygenase (LOX) and epoxygenase. Lipoxygenases are iron-containing dioxygenase with peroxidase activity involved in the synthesis of mediators in inflammatory, atherosclerotic, and carcinogenic processes. RSV and its oxidized form can act as inhibitors of the dioxygenase activity of lipoxygenase while maintaining the hydroperoxidase activity of lipoxygenase [reviewed in (Aggarwal and Shishodia, 2006; Pervaiz, 2003, 2004)].

Multiple reports show that RSV is able to inhibit cyclooxygenase (COX) expression and/or activity; specifically COX-2 (Bhat and Pezzuto, 2002; Surh et al., 2001; Aggarwal and Shishodia, 2006; Cuendet and Pezzuto, 2000; Murias et al., 2004). High concentrations of RSV have been shown to trigger apoptosis via decreases in expression and activity of COX and in the production of the COX metabolites, prostaglandin E2 and F2alpha (PGE2 and PGF2α), as well as affecting cell survival pathways such as the PI3K/PKB/AKT, p53-NFκB, and mitogen activated protein kinases (MAPKs) (Aggarwal et al., 2004; Kundu et al., 2004; Sexton et al., 2006; Tang et al., 2006). Moreover, RSV inhibits the production of free radical oxygen species (ROS) via NADPH oxidase and this correlates with the inhibition of the PI3K activity representing an anti-inflammatory mechanism (Ahmad et al., 2004; Poolman et al., 2005).

More recent studies on a variety of species, including mammals, showed that both RSV and caloric restriction increased silent information regulator 2/sirtuin 1 (SIRT) activity, which resulted in an increase in life span/cell survival. SIRTs belong to the class III histone/protein deacetylases, which are implicated in calorie restriction, aging, neurodegenerative diseases and inflammation (Cosgrove et al., 2006; Leibiger and Berggren, 2006; Longo and Kennedy, 2006). The mammalian SIRT1 (Sir2alpha) physiologically interacts with forkhead box O (FOXO) transcription factors 3/4. The activity of FOXO4 is suppressed or enhanced by SIRT1 inhibitor, nicotinamide (NAD), or its activator, RSV, respectively. In response to oxidative stress, FOXO4 accumulates within the nucleus and induces GADD45 expression. These results indicate that mammalian SIRT1 plays a pivotal role in FOXO function via NAD-dependent deacetylation in response to oxidative stress, and thereby may contribute to cellular stress resistance and longevity (Ying, 2006; Kolthur-Seetharam et al., 2006). In this regard, a recent report has shown that middle-aged mice on a high-calorie diet when given RSV, exhibited the physiology of mice on a standard diet, and had a significant increase in overall lifespan (Baur et al., 2006 (Baur et al., 2006). Investigations showed that RSV was able to prevent the effects of the high-calorie diet in 144 out of 153 significantly altered pathways. Furthermore RSV increased insulin sensitivity, reduced insulin-like growth factor-1 (IGF-I) levels, increased AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-gamma co-activator 1 alpha (PGC-1α) activity, increased mitochondrial numbers, and improved motor functions (Baur et al., 2006). This is further corroborated by a recent report documenting that mice given RSV showed an increase in their aerobic capacity (Lagouge et al., 2006). RSV's effects correlated with induction of genes involved in oxidative phosphorylation and mitochondrial biogenesis, brought about by a decrease in PGC-1α acetylation and an increase in PGC-1α activity, not seen in SIRT-/- mouse embryonic fibroblasts (MEFs). Of note, RSV treatment protected mice against diet-induced-obesity and insulin resistance.

Mitochondria, electron transport chain, and bioenergetics

As the powerhouse of ATP production, the mitochondria respond to the cell's energy needs via the process of oxidative phosphorylation involving the mitochondria respiratory chain located in the mitochondria inner membrane. This is facilitated by extrusion of protons (H^+) from the mitochondria matrix, generating the mitochondria trans-membrane potential and the pH gradient, which together give rise to the proton motive force. Disruption in this chemi-osmotic coupling by inhibiting the re-entry of H^+ back into the matrix through the F_1F_0 ATPase, results in the cessation of ATP synthesis; F_1F_0 ATPase couples the transfer of H^+ down the electrochemical gradient to generate ATP. Evidence is emerging to implicate the Bcl-2 family of proteins in the regulation of mitochondrial bioenergetics. To that end, overexpression of Bcl-2 was shown to shift the Ca^{2+} threshold of cells to one which allows a large amount of Ca^{2+} to accumulate before apoptosis is triggered by deactivating the K^+/ATP exchanger (Cheng et al., 2006). Although the exact mechanism of RSV's ability to trigger depolarization of the mitochondria membrane is not presently well defined, early work suggested that RSV could affect the F-type ATPase to decrease the activity of the mitochondria complex III and reduce ROS generation; piceatannol and RSV inhibited the F-type ATPase by targeting the F_1 sector located on the inner membrane of mitochondria as well as plasma membrane of normal endothelial cells and several cancer cell lines (Zheng and Ramirez, 1999; Zheng and Ramirez, 2000). Other data indicated ROS scavenging activity of RSV, thus lending support to its anti-oxidant property. However, more recent work has shed light on the ability of RSV to function both as a pro-oxidant as well as an anti-oxidant depending upon the concentration and the cell type. To that

end, we demonstrated the ability of RSV to trigger apoptosis in human tumor cells at relatively high concentrations (Clement et al., 1998; Lee et al., 2006), whereas exposure of cells to low (non-cytotoxic) doses resulted in a totally opposite effect, *i.e*., inhibition of death signaling (Ahmad et al., 2004). The latter was attributed to the ability of low concentrations of RSV to create a slightly pro-oxidant intracellular milieu (Ahmad et al., 2003, 2004) that favored cell proliferation and/or inhibited apoptotic execution via mechanisms alluded to in our previous publications (Clement et al., 2003; Pervaiz et al., 1999, 2001). Along similar lines, RSV has been reported to inhibit Akt/PKB phosphorylation and downstream proliferative signaling by targeting the survival kinase, phosphatidylinositol 3-kinase (PI3K). As Akt/PKB is involved in glucose uptake and metabolism, this effect of RSV reduces glycolysis and cellular metabolism. Furthermore, RSV-mediated inhibition of downstream effectors of the PI3K pathway, such as p70 S6K, and S6 ribosomal protein has been recently linked to the arrest of cells in the Go/G1 phase of the cell cycle (Faber et al., 2006). These results are the first to link RSV-induced growth arrest to its effect on the glycolytic pathway.

RSV and mitochondria-mediated cell death

In addition to the pivotal role in ATP synthesis and energy provision for cellular metabolism, the mitochondria play a central role in apoptotic execution. In the mitochondrial-dependent death signaling or intrinsic death pathway, translocation of proteins to and from the mitochondria provides the amplification factors to fuel apoptotic signaling. The intrinsic death pathway is almost obligatory in drug-induced apoptosis, and hence the problem of drug resistance in tumors where this pathway is shut down by the over-expression of anti-apoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-xL (Alves et al., 2006; Galonek and Hardwick, 2006). Recruitment of this pathway is brought about by the translocation of pro-apoptotic proteins of the Bcl-2 family, such as Bax and Bak, thus inducing mitochondrial outer membrane permeablization (MOMP) (Green and Kroemer, 2004). This has a functional impact on mitochondrial morphology as well as physiology, such as dissipation of the trans-membrane potential, loss of the protomotive gradient, leakage of electrons onto oxygen resulting in the generation of free radicals, potentially damaging to the lipid rich milieu of the mitochondrial membranes. The end result is the egress of proteins such as cytochrome C, Smac/DIABLO, caspases, and apoptosis inducing factor (AIF) to the cytosol, where they function in diverse ways to amplify apoptotic signaling (Chipuk et al., 2006).

Although exposure to high concentrations of RSV has been shown to trigger apoptotic death in a variety of models [reviewed in (Pervaiz, 2004)], with a direct or indirect involvement of the intrinsic death pathway, the direct impact of RSV on pathways involved in the induction of MOMP are beginning to be understood. For example, RSV treatment of prostate carcinoma cell line, LNCaP, resulted in a significant loss of mitochondrial membrane potential, inhibition in the protein level of anti-apoptotic Bcl-2, and increase in pro-apoptotic members of the Bcl-2 family, *i.e*., Bax, Bak, Bid, and Bad (Aziz et al., 2006). In a recent communication, our work has implicated caspase 6-mediated cleavage of lamin A in RSV-induced apoptosis of colorectal carcinoma cells, which could be activated in the absence of Bax or p53 (Lee et al., 2006). Along similar lines, earlier reports have demonstrated Bax-dependent as well as Bax-independent induction of apoptosis by RSV in tumor cells (Lu et al., 2001; Mahyar-Roemer et al., 2002).

Interestingly, besides being a strong apoptotic stimulus, RSV in some systems and at low concentrations also exhibits strong protective effects in terms of inhibiting cell death and oxidative damage of tissues/cells. This is supported by the inhibition of (a) oxidized LDL-induced cytotoxicity, (b) mitochondrial trans-membrane potential drop, (c) egress of cytochrome c upon apoptosis induction, (d) cytosolic pH drop, (e) intracellular H_2O_2 production, (f) mitochondrial translocation of Bax, and (g) activation of caspases such as caspase 3 and 9 (Ahmad et al., 2004; Ou et al., 2006).

These paradoxical effects of RSV on cell survival and death pathways have triggered enormous interest, both from the standpoint of its utility as a chemopreventive or chemotherapeutic agent in a variety of disease models, cancer in particular, as well as from the paradoxical perspective of protecting tissues/organs from oxidative damage/degeneration and in disease states where cell death is abnormally amplified, such as Alzheimers disease and HIV/AIDS. Corroborating the latter are its emerging neuroprotective activity as well as the ability to retard aging process in mice (Ingram et al., 2006).

Remarks

Considering the pleiotropic effects of RSV and its myriad intracellular targets it is not surprising that there are a variety of outcomes depending upon the duration and dose of treatment. Future studies are likely to focus on a deeper understanding of the intricate biology of this remarkable compound and its analogues, with potential implications for its use in the clinical settings.

Acknowledgements S.P. is supported by the National Medical Research Council and the Biomedical Research Council, Singapore, as well as the Academic Research Fund, NUS, Singapore. A.L.H is supported by the Academic Research Fund, NUS, Singapore.

Bibliography

Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y (2004) Anticancer Res 24:2783–840

Aggarwal BB, Shishodia S (2006) Biochem Pharmacol 71:1397–1421

Ahmad KA, Clement MV, Hanif IM, Pervaiz S (2004) Cancer Res 64:1452–1459

Ahmad KA, Clement MV, Pervaiz S (2003) Ann N Y Acad Sci 1010:365–373

Alves NL, van Lier RA, Eldering E (2006) Trends Immunol

Aziz MH, Nihal M, Fu VX, Jarrard DF, Ahmad N (2006) Mol Cancer Ther 5:1335–1341

Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA (2006) Nature

Baur JA, Sinclair DA (2006) Nat Rev Drug Discov 5:493–506

Bhat KP, Pezzuto JM (2002) Ann N Y Acad Sci 957:210–229

Cahuzac N, Baum W, Kirkin V, Conchonaud F, Wawrezinieck L, Marguet D, Janssen O, Zornig M, Hueber AO (2006) Blood 107:2384–2391

Cheng WC, Berman SB, Ivanovska I, Jonas EA, Lee SJ, Chen Y, Kaczmarek LK, Pineda F, Hardwick JM (2006) Oncogene 25:4697–4705

Chipuk JE, Bouchier-Hayes L, Green DR (2006) Cell Death Differ 13:1396–1402

Clement MV, Hirpara JL, Chawdhury SH, Pervaiz S (1998) Blood 92:996–1002

Clement MV, Hirpara JL, Pervaiz S (2003) Cell Death Differ 10:1273–1285

Cosgrove MS, Bever K, Avalos JL, Muhammad S, Zhang X, Wolberger C (2006) Biochemistry 45:7511–7521

Cuendet M, Pezzuto JM (2000) Drug Metabol Drug Interact 17:109–157

Dimanche-Boitrel MT, Meurette O, Rebillard A, Lacour S (2005) Drug Resist Updat 8:5–14

Faber AC, Dufort FJ, Blair D, Wagner D, Roberts MF, Chiles TC (2006) Biochem Pharmacol 72:1246–1256

Fulda S, Debatin KM (2005) Eur J Cancer 41:786–798

Galonek HL, Hardwick JM (2006) Nat Cell Biol 8:1317–1319

Gautam SC, Xu YX, Dumaguin M, Janakiraman N, Chapman RA (2000) Bone Marrow Transplant 25:639–645

Gosslau A, Chen M, Ho CT, Chen KY (2005) Br J Cancer 92:513–521

Green DR, Kroemer G (2004) Science 305:626–629

Henkler F, Behrle E, Dennehy KM, Wicovsky A, Peters N, Warnke C, Pfizenmaier K, Wajant H (2005) J Cell Biol 168:1087–1098

Ingram DK, Zhu M, Mamczarz J, Zou S, Lane MA, Roth GS, de Cabo R (2006) Aging Cell 5:97–108

Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM (1997) Science 275:218–220

Kolthur-Seetharam U, Dantzer F, McBurney MW, de Murcia G, Sassone-Corsi P (2006) Cell Cycle 5:873–877

Kopp P (1998) Eur J Endocrinol 138:619–620

Kubo M, Kimurta Y, Shin H, Haneda T, Tani T, Namba K (1981) Shoyakugaku Zasshi 35:58–61

Kundu JK, Chun KS, Kim SO, Surh YJ (2004) Biofactors 21:33–39

Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J (2006) Cell

Langcake P, Pryce RJ (1976) Physiol Plant Pathol 9:77–86

Lee SC, Chan J, Clement MV, Pervaiz S (2006) Proteomics 6:2386–2394

Leibiger IB, Berggren PO (2006) Nat Med 12:34–36; discussion 36

Longo VD, Kennedy BK (2006) Cell 126:257-68

Lu J, Ho CH, Ghai G, Chen KY (2001) Carcinogenesis 22:321–328

Mahyar-Roemer M, Kohler H, Roemer K (2002) BMC Cancer 2:27

Murias M, Handler N, Erker T, Pleban K, Ecker G, Saiko P, Szekeres T, Jager W (2004) Bioorg Med Chem 12:5571–5578

Nonomura S, Kanagawa H, Makimoto A (1963) Yakugaku Zasshi 83:988–990

Ou HC, Chou FP, Sheen HM, Lin TM, Yang CH, Huey-Herng Sheu W (2006) Clin Chim Acta 364:196–204

Pervaiz S (2003) Faseb J 17:1975–1985

Pervaiz S (2004) Drug Resist Updat 7:333–344

Pervaiz S, Cao J, Chao OS, Chin YY, Clement MV (2001) Oncogene 20:6263–6268

Pervaiz S, Ramalingam JK, Hirpara JL, Clement MV (1999) FEBS Lett 459:343–348

Poolman TM, Ng LL, Farmer PB, Manson MM (2005) Free Radic Biol Med 39:118–132

Sexton E, Van Themsche C, Leblanc K, Parent S, Lemoine P, Asselin E (2006) Mol Cancer 5:45

Siemann EH, Creasy LL (1992) An J Eno Vitic 43:49–52

Soleas GJ, Diamandis EP, Goldberg DM (1997) Clin Biochem 30:91–113

Su JL, Lin MT, Hong CC, Chang CC, Shiah SG, Wu CW, Chen ST, Chau YP, Kuo ML (2005) Carcinogenesis 26:1–10

Surh YJ, Chun KS, Cha HH, Han SS, Keum YS, Park KK, Lee SS (2001) Mutat Res 480–481:243–268

Tang HY, Shih A, Cao HJ, Davis FB, Davis PJ, Lin HY (2006) Mol Cancer Ther 5:2034–2042

Velthuis JH, Rouschop KM, De Bont HJ, Mulder GJ, Nagelkerke JF (2002) J Biol Chem 277:24631–24637

Wang Y, Wang B, Cheng J, Yang L, Liu ZL, Balan K, Pantazis P, Wyche JH, Han Z (2005) Biochem Pharmacol 69:249–254

Ying W (2006) Front Biosci 11:3129–3148

Zheng J, Ramirez VD (1999) Biochem Biophys Res Commun 261:499–503

Zheng J, Ramirez VD (2000) Br J Pharmacol 130:1115–1123