The potential of resveratrol against human gliomas

Nicoletta Gagliano[a], Giancarlo Aldini[b], Graziano Colombo[c], Ranieri Rossi[d], Roberto Colombo[c], Magda Gioia[a], Aldo Milzani[c] and Isabella Dalle-Donne[c]

There is growing interest in dietary phytochemicals as potential cancer chemopreventive agents. Resveratrol (3,4′,5-trihydroxy-*trans*-stilbene), a naturally occurring phytoalexin that is present in grapes, red wine, berries and peanuts, has been studied extensively for its ability to interfere with multistage carcinogenesis. Resveratrol is known to have antioxidant, anti-inflammatory and antiproliferative effects on a variety of cancer cells *in vitro* and in various animal models. However, the effect(s) of resveratrol *in vivo* on humans are still controversial. This study discusses current knowledge with regard to the effects of resveratrol in relation to its potential as a chemopreventive and/or chemotherapeutic molecule against human gliomas.

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Departments of [a]Human Morphology and Biomedical Sciences 'Cittá Studi', [b]Pharmaceutical and Toxicological Chemistry 'Pietro Pratesi', [c]Biology, Università degli Studi di Milano, Milan and [d]Department of Evolutionary Biology, University of Siena, Siena, Italy

Correspondence to Dr Nicoletta Gagliano, PhD, Department of Human Morphology and Biomedical Sciences 'Cittá Studi, Università degli Studi di Milano, School of Medicine, Extracellular Matrix Laboratory, Via Fratelli Cervi 93, 20090 Segrate, Milan, Italy
Tel: +39 2 50330462; fax: +39 2 50330452;
e-mail: nicoletta.gagliano@unimi.it

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Introduction

Gliomas are the most malignant of primary tumours that affect the brain and nervous system and carry the worst clinical prognosis in both adults and children. Effective therapy for such tumours is limited by their resistance to conventional treatments. Invasive behaviour is a critical prognostic factor for gliomas, and contributes to the failure of current therapies. With rare exceptions, gliomas do not metastasize outside the brain to systemic organs, being locally invasive [1] because of the absence of blood vessel wall invasion, together with the absence of lymphatics within the brain.

Gliomas arise from the neoplastic transformation of astrocytes, oligodendrocytes and microglia, and are categorized according to the type of glial cell from which they stem. The World Health Organization (WHO) has developed a classification scheme that grades gliomas from the least malignant to the most aggressive tumour type, that is, from grades I–IV (sometimes referred to as low vs. high grade). The most aggressive tumours have been assigned grade IV or glioblastoma multiforme. Therefore, according to the WHO, the three main types of gliomas, distinguished by their histological features, are oligodendrogliomas, mixed oligoastrocytomas and astrocytomas [2].

Oligodendrogliomas arise from oligodendrocytes and represent 30% of all adult gliomas. They infiltrate the grey and white matter in a diffuse manner, and range from benign and slow-growing to malignant and rapidly progressing. Mixed oligoastrocytomas arise from a mixture of astrocytes and oligodendrocytes, and tumours range from slow-growing to malignant and rapidly progressive. Astrocytomas arise from astrocytes and constitute 65–70% of all gliomas. Among astrocytomas, four major grades are defined: low-grade astrocytomas, or WHO grades I and II and high-grade or malignant astrocytomas, or WHO grades III and IV [3]. Pilocytic astrocytomas (WHO grade I), the most frequent brain tumours of children, are slow-growing lesions that rarely undergo progression to malignant gliomas, and tend to be treated effectively by surgical resection. Diffuse-type (or low-grade) astrocytomas (WHO grade II) are frequent in adults between 30 and 40 years of age. They account for 25% of all gliomas and are slower-growing tumours, characterized by their moderate cellularity and level of infiltration, but they can progress to anaplastic astrocytoma and then glioblastoma, and eventually to malignant grades. Anaplastic astrocytomas (WHO grade III) are highly malignant, being characterized by increased cellularity, pleomorphism and nuclear atypia. They show an increased tendency to progress to glioblastoma, infiltrate through neighbouring tissues, and are often diagnosed when they have become large and are having mass effects on adjacent structures.

Glioblastoma multiformes (WHO grade IV) are the most common and highly malignant gliomas, being rapidly growing and aggressive [2]. They are composed of poorly differentiated pleomorphic cells with very high mitotic activity, and are also highly heterogeneous in gross and microscopic appearance. Glioblastomas infiltrate diffusely into regions of the normal brain, making total surgical removal very difficult; in fact, after surgical resection, the residual pool of invasive cells gives rise to a recurrent tumour that, in more than 90% of cases, develops immediately adjacent to the resection margin or within the resection cavity. As a

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consequence, the prognosis for patients with glioblastoma remains approximately 1 year despite aggressive surgical resection, radiotherapy and chemotherapy [4]. Glioma resistance to radiation and chemotherapy is responsible for poor patient prognosis, and has been attributed to a variety of mechanisms including altered antioxidant enzyme expression [5–8].

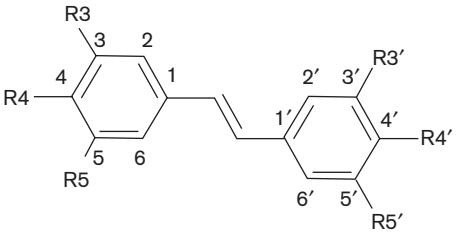
Resveratrol (3,4′,5-trihydroxy-*trans*-stilbene, RSV) is a naturally occurring polyphenolic compound abundant in grapes, peanuts, red wine, pines and other leguminosae family plants in response to injury, ultraviolet irradiation and fungal attack. The anticarcinogenesis activity of RSV was first shown in a pioneering study by Jang and Pezzuto [9], who reported that RSV was effective in all the three major stages (initiation, promotion and progression) of carcinogenesis [10]. RSV suppresses the proliferation of a variety of human cancer cells *in vitro* [11,12], including glioma cells [13].

Resveratrol

RSV is a potent antioxidant because of its ability to scavenge free radicals, spare and/or regenerate endogenous antioxidants, that is, glutathione (GSH) and α-tocopherol, and to promote the activities of a variety of antioxidant enzymes. RSV scavenges lipid peroxyl radicals through an H-transferring mechanism (Fig. 1), and $HO^{\bullet}$ and $O_2^{-\bullet}$ radicals [14]. Structure–activity relationship studies of RSV and its analogues revealed that the 4′-hydroxy group is essential for the radical scavenging activity, and that it acts synergistically with the 3- and 5-OH groups. The antioxidant property of RSV is also attributed to its ability to promote the activities of a variety of antioxidant enzymes. When intraperitoneally administered in rats, it was found to dose-dependently increase superoxide dismutase (SOD), catalase and peroxidase activities in the brains of healthy rats [15]. The long-term exposure of human lung fibroblasts to RSV results in a highly specific upregulation of MnSOD [16]. In human lymphocytes, RSV (10–100 μmol/l) increased GSH levels and the activity of glutathione peroxidase, glutathione-S-transferase and glutathione reductase (GR) [17].

Depending on the concentration and cell type, RSV can also act as a prooxidant molecule [14], this effect being Cu(II)-dependent. Such a prooxidant effect could be an important action mechanism for its anticancer and proapoptotic properties. Compared with normal cells, cancer cells have been shown to contain elevated levels

Fig. 1



Compound	R3	R4	R5	R3′	R4′	R5′
Resveratrol	OH	H	OH	H	OH	H
4′-hydroxy-*trans*-stilbene	H	H	H	H	OH	H
3,5-dihydroxy-*trans*-stilbene	OH	H	OH	H	H	H
3,4-dihydroxy-*trans*-stilbene	OH	OH	H	H	H	H
3,4,4′-trihydroxy-*trans*-stilbene	OH	OH	H	H	OH	H
3,4,5-trihydroxy-*trans*-stilbene	OH	OH	OH	H	H	H
3′-methyl-resveratrol	OH	H	OH	CH_3	OH	H
3′,5′-dimethyl-resveratrol	OH	H	OH	CH_3	OH	CH_3

Structure of resveratrol and its hydroxyl and methyl derivatives.

of copper, and hence might be more sensitive to the prooxidant and cell-damaging effects of RSV. Therefore, DNA damage induced by RSV in the presence of Cu(II) might be an important pathway through which cancer cells can be killed while normal cells survive [18].

Bioavailability of resveratrol

The bioavailability of RSV and its kinetics are relevant factors to be taken into account when considering its possible use as a chemopreventive agent. An in-vivo study performed in gerbils showed first the ability of RSV to cross the blood–brain barrier and incorporate into brain tissue [19], supporting its possible use as a therapeutic chemopreventive agent in brain tumours.

Notwithstanding numerous attempts, only limited information on RSV bioavailability in humans is available [20,21]. The oral ingestion of ^{14}C-labelled RSV by human volunteers led to a concentration of 2 μmol/l of total RSV and a plasma half-life ranging from 6.5 to 14.9 h; on the basis of the urinary excretion data, the absorption of RSV seemed to be at least 70% [22]. In humans, orally administered RSV is rapidly metabolized in the liver by phase-II detoxification enzymes, accounting for its predominant urine excretion [22]. This study has shown a high absorption but a very low bioavailability of RSV after oral administration in humans [22]. Dietary RSV (up to 25 mg) is rapidly adsorbed and predominantly present in the plasma as glucoronide and sulphate conjugates; in addition, its metabolism is significantly inhibited by other polyphenols owing to competitive reactions with metabolizing phase-II enzymes [21], resulting in an increased concentration of the free form. Despite this, the free aglycone is almost undetectable in human plasma. Although several studies were performed in preclinical models [11,23], the large amount of data on anticancer activity of RSV is based on studies on cultured cell lines, where the molecule is given as aglycone, a form almost absent in plasma and urine, and therefore they should be interpreted with extreme caution [21].

Toxicity of resveratrol

In rats and mice, RSV toxicity is minimal, and even actively proliferating tissues (e.g., bone marrow, gastrointestinal tract) are not adversely affected. No renal toxicity was observed in rats fed with a dose of 300 mg RSV/kg/day for 4 weeks (equivalent to 21 g of RSV for an adult man of 70 kg) [24]. Histopathological examination of the organs obtained at autopsy of rats administered orally for 28 days at a dose of 20 mg/kg/day (this dose is 1000 times the amount consumed by a 70-kg person taking 1.4 g of RSV/day) did not reveal any alterations [25]. Administration of 4 g of RSV/kg/day for 28 days in mice (equivalent to 280 g RSV/day for an adult man of 70 kg) lacked oncogenicity, although it caused mild anaemia and an increase in liver weight and serum cholesterol [26]. The absence of symptoms and the normal appearance of vital organs in rats and mice suggested that RSV is barely toxic even under the conditions that have been described. Indeed, the minimal toxicity in animals has allowed RSV to enter human studies sponsored by the National Cancer Institute (NIH, USA) aimed at cancer prevention using healthy volunteers. However, the metabolism in rats and mice differs substantially from that in humans, which has not yet been established, and therefore precaution should prevail. Moreover, a very recent study aimed at evaluating oxidative damage biomarkers during ageing in F2 hybrid mice and the eventual protection by long-term oral intake of RSV (14.09 ± 3.4 mg/l in drinking water for 6 or 12 months) showed that chronic treatment with RSV consistently attenuated oxidative damage in tissues in which age-related oxidative damage accumulation was prominent, but also suggested that a 12-month RSV intake may induce nephrotoxicity [27].

A phase I study of oral RSV (single doses of 0.5, 1, 2.5, or 5 g) was conducted in 10 healthy volunteers per dose level, and RSV and six metabolites were identified in plasma and urine [28]. Consumption of RSV did not cause serious adverse events. The peak plasma levels of RSV at the highest dose were 539 ± 384 ng/ml (2.4 μmol/l, mean ± SD), whereas the peak levels of two monoglucuronides and RSV-3-sulphate were three- to eight-fold higher. As cancer chemopreventive effects of RSV in cultured cells require levels of at least 5 μmol/l, these results suggest that the consumption of high-dose RSV might be insufficient to elicit systemic levels commensurate with cancer chemopreventive efficacy. The high systemic levels of RSV conjugate metabolites would warrant investigation of their potential cancer chemopreventive properties [28].

Oxidative stress in glioma cells and effects of resveratrol

Oxidative stress is a situation in which the cellular homeostasis is altered because of excessive production of reactive oxygen species (ROS) and/or impairment of cellular antioxidant defence(s), leading to oxidative damage to macromolecules and to a disruption of redox signalling and control [29]. A number of validated biomarkers of oxidative stress/damage are available [30,31].

The brain is particularly susceptible to oxidative stress owing to its high consumption of oxygen and large quantities of polyunsaturated fatty acids. ROS interfere with the expression of a number of genes and signal transduction pathways, and are thus involved in the process of carcinogenesis [32,33].

Cultured glioma cells adapt to oxidative stress by activating several pathways that protect them from oxidative stress-induced apoptosis. A highly specific genome-scale study, performed to identify genes differentially expressed in cultured glioma cells versus normal

brain tissue, revealed that aldo-keto reductase family 1, member A1, *AKR1A1* and member C1, *AKR1C1*, which protect cells by efficiently detoxifying and reducing aldehydes and ketones, as well as several antioxidant genes, such as peroxiredoxin (Prx) I and thioredoxin reductase 1, and *GAPD* (glyceraldehyde-3-phosphate dehydrogenase) and *MCP1* [chemokine (C-C motif) ligand 2], are upregulated in cultured glioma cells [34]. GAPD mediates the hydrogen peroxide (H_2O_2)-dependent activation of phospholipase D2, which protects against apoptosis [35]. The constitutive activation of nuclear factor-kappa B (NF-κB) was found in cultured glioma cells and glioblastoma surgical samples [36,37]. Activated NF-κB induces *MCP1* and protects against apoptosis by regulating several antiapoptotic proteins [38].

The activities of Cu/ZnSOD and MnSOD were found to be decreased in human meningiomas, low-grade astrocytomas, high-grade astrocytomas and medulloblastomas, whereas the catalase levels were significantly increased in ascending order in high-grade astrocytomas, low-grade astrocytomas and meningiomas [39].

Large differences were found in the expression of antioxidant enzymes in different glioma cell lines [5]. MnSOD activity was generally higher in human glioma cells than in rat glioma cells and relatively higher than in other tumour types, the increased activity being a result of the high levels of expression of MnSOD mRNA and protein. Heterogeneous expression of MnSOD was present in individual glioma cell lines, and was attributed to subpopulations or cells at different differentiation stages [5]. Catalase expression in several human and rat glioma cell lines was correlated with their resistance to the chemotherapeutic agent, carmustine [1,3-bis(2-chloroethyl)-1-nitrosourea] [5], a DNA-alkylating agent used clinically in the treatment of gliomas, which acts in part through oxidative stress by inhibiting GR.

Gliomas seem to constitutively overexpress catalase when compared with their normal cell counterpart, the astrocytes and catalase activity was more than two-fold greater in the glioma cells than in the astrocytes [6]. 36B10 rat glioma cells exhibited a higher level of Cu/ZnSOD enzymatic activity than did the normal astrocytes. In contrast, glutathione peroxidase activity values in the glioma cells were significantly lower than those observed in normal astrocytes [6].

A recent study [7] observed that both catalase protein and enzymatic activity are elevated in a number of human and rat glioma cell lines when compared with normal astrocytes, confirming earlier in-vitro and in-vivo data [6,39]. In addition, 2-Cys Prx levels were also elevated constitutively in rat 36B10 glioma cells when compared with primary rat astrocytes. Moreover, Prx I and II expressions were increased after catalase inhibition [7]. Although inhibiting catalase expression and activity resulted in increased oxidative stress in 36B10 glioma cells, this did not lead to any apparent change in glioma cell viability, but, notwithstanding the increased Prx expression, apparently sensitized these cells to ionizing radiation and H_2O_2 [7].

It has recently been shown that rat and human glioma cells overexpress 2-Cys Prx II, a cytosolic peroxide and peroxynitrite-scavenging antioxidant enzyme, when compared with their normal cell counterpart, the astrocytes [8]. Decreasing Prx II increased intracellular ROS in 36B10 rat glioma cells; extracellular levels of H_2O_2 were also increased in both C6 and 36B10 cells. Moreover, knocking down Prx II leads to an increase in radiosensitivity at clinically relevant doses as low as 1 Gy, and to an increased sensitivity to oxidative stress. This increased sensitivity is a result, in part, of a decline in GR activity lowering intracellular GSH, and of alterations in cell cycle distribution. Indeed, decreasing Prx II expression in glioma cells also reduced clonogenic cell survival after exposure to ionizing radiation and H_2O_2 [8]. These data suggest that overexpression of Prxs protects cancer cells from oxidative stress. As ROS production is elevated in cancer cells, overexpression of Prx II may provide additional protection against ROS generated from the increased metabolism of glioma cells.

Proteomic analysis showed that expression of both Prx I and Prx VI was further increased in high-grade gliomas when compared with low-grade gliomas, suggesting a prognostic role for Prx I and Prx VI [40]. However, Prx II expression was generally conserved during progression from low- to high-grade glioma [40]. These data suggest that overexpression of Prx II may provide additional defence against ROS contributing to glioma cell resistance to anticancer therapies.

A recent study aimed to characterize markers of oxidative status and mitochondrial function in the centre versus the periphery of human fresh glioma samples (gross total resections) [41]. The tumour periphery exhibited a higher respiratory rate and fewer antioxidant systems than tumour the centre: significantly higher cellular levels of GSH (expressed per gram of fresh tissue), MnSOD activity and GSH/GSSG ratio were found in central glioma areas when compared with the peripheral areas. The increase in antioxidant systems in the central region could be considered an adaptative mechanism by which cancer cells counteract chronic oxidative stress, and might be responsible for tumour resistance to chemotherapy and radiotherapy [41].

RSV protects rat primary cortical astrocyte cultures against acute H_2O_2-induced oxidative stress by inhibiting ROS production, improving glutamate uptake activity, increasing GSH content and stimulating S100B secretion [42]. In U251 glioma cells, treatment with RSV led to growth inhibition, induction of apoptosis and G_0/G_1-phase cell

cycle arrest [43]. A recent study tested the effect of combination treatment with RSV and sulforaphane, when both were used at low concentrations (25 μmol/l), on cell proliferation, migration and death in human U251 glioma cells. Combination treatment with resveratrol and sulforaphane inhibited cell proliferation and migration, reduced cell viability, induced lactate dehydrogenase release, decreased prosurvival Akt phosphorylation and increases caspase-3 activation [44]. RSV, in combination with quercetin (a structurally related, naturally occurring polyphenol), decreased the cell number in four rat and human glioma cell lines, but not in rat astrocytes. Low doses of RSV (10 μmol/l) or quercetin (25 μmol/l) separately had no effect on apoptosis induction, but had a strong effect on caspase 3/7 activation when administered together [45]. In contrast, high doses of RSV ($\geq$ 50 μmol/l) induced necrosis, apoptosis and cell cycle arrest at early time points. RSV and quercetin chronically administered presented a strong synergism in inducing senescence-like growth arrest [45]. These results suggest that the combination of polyphenols can potentialize their antitumoral activity. Altogether, the results of the most recent studies suggest that the use of RSV in combination with other bioactive food components, such as quercetin and sulforaphane, might be a viable approach for the treatment of human glioma.

Anti-inflammatory properties of resveratrol

Inflammatory stimuli contribute to the progression of cancers [46], and the close relationship between inflammation and cancer is suggested by the identification of a number of inflammatory conditions that predispose humans to cancer [47].

RSV can be compared with nonsteroidal anti-inflammatory drugs. Considerable evidence shows anti-inflammatory properties of RSV, including inhibition of ROS production in neutrophils, monocytes and macrophages. The release of various cytokines from macrophages and lymphocytes, such as IL-12, IFN-γ, IL-2 and TNF-α, as well as the expression of inducible nitric oxide synthase and the release of nitric oxide, in stimulated macrophages, has been shown to be inhibited by RSV. The strong anti-inflammatory properties of RSV are partly a result of its inhibitory effects on enzyme systems involved in the synthesis of pro-inflammatory mediators, such as the cyclooxygenase (COX) and the lipoxygenase pathways. Downregulation of the expression of proinflammatory mediators, including COX, is also mediated through RSV inhibitory effects on transcription factors that regulate the expression of such inflammatory mediators, in particular NF-κB and activator protein-1 [48].

COX catalyses the conversion of arachidonic acid to modulators of inflammation such as prostaglandins, which can stimulate tumour cell growth by acting on carcinogen metabolism, tumour cell proliferation, angiogenesis, immunosuppression and metastatic potential. While COX-1 is constitutively expressed, COX-2 has received much attention as a target for chemopreventive agents, because it is constitutively overexpressed in inflammation and several types of human cancers, and seems to control many cellular processes [49]. COX-2 overexpression has been documented in human gliomas [50,51] and correlated with poor survival for all malignant gliomas, the strongest correlation being with glioblastoma multiforme [50]. This study also showed increasing enzyme expression with increasing histologic grade, and found COX-2 expression to be a strong predictor of survival, independent from other variables. It was suggested that COX-2 is most relevant to the postinitiation phase of tumour promotion through mechanisms such as angiogenesis and the subversion of the host antitumour immune response. On account of its role in carcinogenesis, apoptosis and angiogenesis, COX-2 is thus an excellent target for developing new drugs with selectivity for prevention and/or treatment of human cancers. Development of selective COX-2 inhibitors has been successfully documented [52], and studies performed to address possible antineoplastic activity in humans successfully established that COX-2 inhibitors can be considered as valuable therapeutic agents against various human cancers, including gliomas [53–57]. However, the long-term use of COX-2 selective inhibitors showed severe cardiovascular toxicity, precluding their use as prophylactic agents [58]. Therefore, their use in cancer prevention and therapy is currently questionable, suggesting that further development of novel COX-2 selective agents is required [52,59].

RSV specifically inhibited COX-1 *in vitro* and, although there were conflicting results [10], some authors reported that it also inhibited *in vitro* both COX-2 activity and gene expression [10,60,61]. Therefore, RSV-induced anti-inflammatory activity might be explained by the inhibition of COX-1 and COX-2. These findings suggested that RSV can be considered a potential chemopreventive agent inhibiting tumour promotion, and its inhibitory effect on COX activity has been suggested as one of the possible molecular mechanisms responsible for its anticancer activity [48]. As COX promotes angiogenesis, its suppression by RSV represents further inhibitory mechanisms of tumour growth. Consistently, to find more selective COX-2 inhibitors, a series of methoxylated and hydroxylated RSV derivatives were synthesized and evaluated for their ability to inhibit both COX isoenzymes by measuring in-vitro prostaglandin E_2 production [62]. The results were then compared with that of RSV and the selective COX-2 inhibitor celecoxib. Hydroxylated, but not methoxylated, RSV analogues showed a high rate of inhibition, the former being the most potent RSV compound with potency comparable to or better than the clinically established celecoxib [62]. The occurrence of a quantitative relationship between chemical structure and biological activity

was evaluated by quantitative SAR analysis, and a high correlation between structural parameters and COX-2 inhibition was found. Thus, hydroxylated RSV analogues showed significantly lower IC_{50} values against COX-2 than celecoxib, which should result in lower doses necessary to achieve the same efficacy in clinical studies [62].

NF-κB is a nuclear transcription factor able to activate the expression of inflammatory and antiapoptotic genes, which is activated by a variety of stimuli, such as carcinogenesis, inflammatory agents and tumour promoters. NF-κB induces overactivation of the COX enzymes and is associated with increased angiogenesis [63,64]. In cancer, NF-κB is constitutively activated and seems to promote tumorigenesis or tumour cell survival. Most carcinogens and tumour promoters indeed activate NF-κB, whereas chemopreventive agents suppress it [38]. Thus, while activation of NF-κB promotes cell survival and proliferation, its downregulation sensitizes the cell to apoptosis. Similarly, most inflammatory agents mediate their effects through the activation of NF-κB, and most anti-inflammatory agents suppress NF-κB activation [48]. Interestingly, such dysregulation was described in gliomas. Elevated NF-κB-related gene expression in actively migrating glioma cells *in vitro* and invading cells *in vivo* suggests a role for NF-κB in migration and invasion [65,66], supporting its role in invasive glioma cell survival. RSV was shown to *in vitro* inhibit both NF-κB and activator protein-1 activation triggered by different stimuli that involve different intracellular signalling pathways [67,68]. In-vitro studies showed that RSV can inhibit nuclear localization of the p65 subunit or the inactivation and degradation of IκBa, which inhibits NF-κB activation by sequestering it in the cytoplasm [69]. Modulation of NF-κB activity, associated with the inhibition of COX-2 expression, is involved in the neuroprotective action of RSV against β-amyloid-induced toxicity in cultured rat astroglioma C6 cells [70]. In fact, β-amyloid is thought to be elaborated as part of an inflammatory process in which activated microglia, the predominant source of COX-2-dependent prostanoids, participate [71].

RSV also elicits its anti-inflammatory effects by inhibiting lymphocyte proliferation by suppressing the expression of CD28/CTLA-4 and CD80 co-stimulatory molecules [72], as well as by inducing both caspase-dependent and caspase-independent apoptosis in activated T-cells, as shown in experimental allergic-encephalomyelitis model in rats [73].

Cell cycle arrest and cell death-inducing properties of resveratrol

The induction of apoptosis is a key mechanism for most antitumour therapies. RSV possesses strong antiproliferative properties in many cultured cancer cell lines, and acts both by arresting cell cycle and by inducing apoptosis [11,12], but the apoptosis-inducing effects of RSV seemed diverse on different tumour cells [11–13,74]. Conflicting results have indeed been obtained with regard to the mechanisms associated with induction of apoptosis by RSV in tumour cells [11,12,48,75–77], among which caspase-3 activation plays a relevant role [43,76,78].

RSV (and some of its analogs) has been shown to interfere with signal transduction pathways, to modulate cell cycle-regulating proteins and to induce apoptosis in multiple cancer cell lines with various mechanisms, including through a p53-dependent pathway [11,12]. The proapoptotic protein nuclear factor p53 plays a key role in protecting a cell from tumorigenesis, halting the cell cycle or initiating apoptosis if the cell is damaged. Mutations in p53 leading to its inactivation have been found in many human cancers, including brain tumours [79].

RSV initiates p53-dependent apoptosis in human cancer cells, including glioma cells [75], through essential binding to plasma membrane integrin αVβ3 [80]. In cultured human glioma cells, RSV-activated protein kinase C and extracellular-signal-regulated protein kinases (ERK1/2) caused COX-2 expression and nuclear COX-2 accumulation, with consequent p53 phosphorylation and apoptosis [75]. This finding seems inconsistent with the previously described anti-neoplastic activity of COX-2 inhibition exerted by RSV. This discrepancy was explained in a recent study [81] showing that COX-2 inhibition can have two different effects on cancer depending on the constitutive or inducible COX-2 expression. In fact, constitutive COX-2 expression is growth-promoting, while inducible nuclear COX-2 expression is a factor that supports RSV-induced p53-dependent apoptosis. A similar effect was elicited by different anti-inflammatory drugs such as flurbiprofen on glioblastoma cells, enhancing COX-2 expression and its complexation with p53, and therefore tumour growth suppression [82].

In C6 rat glioma cells, RSV was shown to exert a significant cell growth inhibition effect in a concentration-dependent and time-dependent manner, inducing apoptosis by increased expression of caspase-3 mRNA and caspase-3 activation [78]. However, another study reported that RSV is not metabolized in C6 rat glioma cells, and accumulates to concentrations that drive the cell to necrosis [76]. RSV can also induce both dose-dependent and time-dependent apoptosis in human glioma U251 and U87 cells [43]. RSV-induced apoptosis requires the activation of caspase-3, and involves the upregulation of proapoptotic Bax expression and its translocation to the mitochondria, increased cytochrome *c* release from mitochondria, and activation of caspase-9. RSV inhibits cell proliferation and induction of G_0/G_1 growth arrest through the suppression of cyclin D1 expression, which may contribute to the apoptotic cell death process [43]. Tumour cell death and the direct activation of the mitochondrial intrinsic apoptotic pathway were induced by RVS during in-vivo neuroblastoma in mice [83].

It has recently been confirmed that RSV induces a delay in cell cycle progression in human glioblastoma U87 cells, both alone and in combination with X-rays, particularly because of a delay during the S phase [84]. Interestingly, this study also examined the involvement of gap junction intercellular communication in this modulation, as gap junctions and gap junction intercellular communication are necessary for normal cell cycling, and play an important role in cell growth control. Connexin 43 (Cx43), the most abundant gap junction connexin in astrocytes and glioma cells, decreases as the grade of glioma/astrocytoma worsens, and an inverse correlation between Cx43 expression and tumour malignancy grade exists [85], suggesting that Cx functions as a tumour suppressor. RSV induces a downregulation of phosphorylated Cx43 [84], thus favouring the open status of the gap junction channel and the maintenance of the gap junction intercellular communication.

Antiangiogenic properties of resveratrol in glioma cells

During carcinogenesis, tumour cells stimulate the formation of new blood vessel networks that sustain the development of cancer by providing oxygen and nutrients to tumour cells [86]; thus, solid tumour (including malignant gliomas) growth depends on angiogenesis [86]. Malignant gliomas are vascular tumours that produce the vascular endothelial growth factor (VEGF), which is an important mediator of angiogenesis. Angiogenesis is very prominent in malignant gliomas, and is related to the amount of secreted VEGF [87,88]. The highest microvascular proliferation and microvascular density have been observed in glioblastomas multiformes, and the lowest in oligodendrogliomas, in which endothelial proliferation is a diagnostic hallmark [87] and significant correlation was observed between the vascularisation and malignancy grade [87].

Antiangiogenic therapies that target VEGF and the VEGF receptor are effective adjuncts to the treatment of solid tumours, including malignant gliomas [89]. RSV inhibits VEGF-induced angiogenesis through the disruption of ROS-dependent *src* kinase activation and subsequent vascular endothelial-cadherin tyrosine phosphorylation [90,91].

Few data are available with regard to the antiangiogenic effect of RSV in glioma cells. RSV was found to suppress VEGF expression in rat RT-2 glioma cells in a concentration-dependent and time-dependent manner, and inhibited the proliferation of human umbilical vein endothelial cells, suggesting that the inhibition of endothelial cells proliferation may contribute to the suppression of vessel formation [92]. The antiangiogenic effect of RSV on glioma was confirmed *in vivo*: the administration of 40 and 100 mg/kg/day of RSV to rats bearing intracerebral gliomas induced by injection of RT-2 glioma cells prolonged the animal survival time and suppressed angiogenesis, as suggested by the decrease in microvessel density [92].

The effects of RSV on angiogenesis in rat gliomas were also confirmed by colour Doppler ultrasound [93]. The rats were subcutaneously inoculated with rat RT-2 glioma cells and treated with RSV (40 mg/kg/day) for 4 weeks. RSV-treated rats had slower tumour growth rates than those of the control group, and tumour blood flow, shown as the colour Doppler vascularity index, tumour microvessel density and tumour size were significantly correlated. Therefore, RSV-suppressed glioma growth was significantly correlated with the inhibition of macroscopic and microscopic angiogenesis [93].

Resveratrol and tumour invasion

Efficient tumour invasion requires degradation of the extracellular matrix (ECM) at the invasion front. Activation of zinc-dependent matrix matrix metalloproteinases (MMPs) is the primary response for the degradation of ECM components.

MMPs are overexpressed in human gliomas, and glioma invasiveness is strongly correlated with the proteolytic activity of MMPs [94,95]. MMP-2 and MMP-9 expression correlates with the progression and degree of malignancy of human gliomas [96,97]. The expression of MMP-1, MMP-11 and MMP-19 has recently been shown to be correlated with the WHO grading of human malignant gliomas [98]. An enhanced expression of these MMPs has been shown in glioblastoma multiformes compared with low-grade astrocytomas or normal brain. The transition from low-grade astrocytomas to glioblastoma multiformes was characterized by a shift of pro-MMP-11 to expression of the active enzyme [98]. In our laboratory, we analyzed the effect of RSV on MMP-2 levels in glioblastoma cells. Our data show that RSV significantly and dose-dependently lowered MMP-2 levels in glioblastoma cells at the mRNA and protein level [13].

MMPs also promote tumour progression through signalling functions [99]. As a consequence, it is pivotal to determine whether an MMP is a possible therapeutic target or, by contrast, an antitarget because it is required for the essential functions of normal tissues. MMP-2, (and MMP-7) was validated as an anticancer drug target in some aggressive tumours [100], and, accordingly, its inhibition by RSV might represent a relevant molecular mechanism of chemoprevention.

During tumour invasion, ECM surrounding malignant glioma undergoes remodelling, also involving secreted protein acidic and rich in cysteine (SPARC) [101], a matricellular glycoprotein that mediates cell-matrix interactions [102]. SPARC is abnormally expressed in several solid cancers, including malignant gliomas. SPARC protein levels correlate with glioma invasion *in vitro* and *in vivo* [103–106]. At the same time, however, intracellular SPARC

is able to decrease tumour growth, in part, by decreasing the proliferation of the tumour cells owing to cell cycle inhibition properties [103]. As SPARC is expressed at low levels in the normal adult brain, but highly expressed in gliomas, SPARC is an attractive target in brain tumour therapy. Increased SPARC expression levels in the microenvironment have been linked to poor glioma patient survival [105], suggesting that the reduction in SPARC expression might have therapeutic benefit. We studied the effect of RSV on intracellular and secreted SPARC expression in glioblastoma cells, to determine whether RSV is able to modulate glioblastoma progression and tumour microenvironment remodeling. Interestingly, our data showed that RSV was able to induce a dose-dependent downregulation of the SPARC gene and secreted protein expression in human glioblastoma cells *in vitro* [13]. This is an interesting finding considering that SPARC can enable glioma cells to survive under the stressful conditions that surround the tumour, also reducing the apoptotic rate [104]. Recent data showing that downregulation of SPARC expression decreased glioma cell survival and invasion further show the role of SPARC in glioma tumour progression [107]. Therefore, SPARC could be an important target of RSV for the design of strategies to restrict glioma invasion. However, further recent data indicate that SPARC modulates glioma growth by increasing glioma matrix and suppressing glioma vascularity through reduction in VEGF expression and secretion [108]. Therefore, a better understanding of SPARC complex signalling mechanisms is important, as inhibiting SPARC alone to decrease invasion could lead to the growth of more vascular and proliferative gliomas (Tables 1 and 2).

Table 1 Some cell lines in which cell cycle-arresting and apoptosis properties of resveratrol have been found

Cell lines	Effect	References
Human epidermoid carcinoma A431 cells	G_1-phase arrest	[73]
Human oesophageal adenocarcinoma Bic-1	G_1-phase arrest	[109]
Myeloma-derived cell lines U266	S-phase arrest	[110]
A375 melanoma cell line	Apoptosis	[111]
Hormone-insensitive DU 145 prostate cancer cells	Apoptosis	[112]
Human B cell chronic leukaemia	Apoptosis	[113]
Human T-cell acute lymphoblastic leukaemia MOLT-4 cells	Apoptosis	[114]
Human uterine cancer cells	Apoptosis	[115]
Leukaemic haematopoietic cells	Apoptosis	[116]
LNCaP prostate cancer cells	Apoptosis	[117]
MDA-MB-231 human breast cancer cells	Apoptosis	[118]
WI38VA cancer cell lines	Apoptosis	[119]

Table 2 Some human cell lines from SNC in which cell cycle-arresting and apoptosis properties of resveratrol have been found

Cell lines	Effect	References
Human neuroblastoma SH-SY5Y cells	S-phase arrest	[120]
Murine neuro-2a neuroblastoma cells	S-phase arrest	[121]
Glioblastoma U373MG, A172 cells	G_1-phase arrest	[122]
Human glioma U251 cells	G_1-phase arrest	[43]
Rat C6 glioma cells	S-phase arrest	[78]

Conclusion

Clinical treatment of gliomas involves multimodality treatment including surgery, radiation and chemotherapy. Inevitably, death ensues owing to local tumour progression and recurrence; therefore, methodologies to sensitize gliomas to anticancer therapies are clearly required. Gliomas are extremely resistant to radiotherapies and chemotherapies, resulting in poor patient survival, owing, in part, to altered expression of antioxidant enzymes. This can offer a survival advantage for cancer cells by reducing the efficacy of anticancer therapies such as ionizing radiation and chemotherapeutics that work through the generation of ROS. As glioblastoma multiforme is refractory to ionizing radiation and chemotherapy, altering the redox environment of these cells might sensitize them to anticancer therapies mediated through oxidative stress [8]. RSV-induced inhibition of catalase and/or inhibition of Prx might represent an additional tool for the current clinical armamentarium that might increase glioma cell kill, leading to improved survival time for patients.

Despite substantial progress in the understanding of the molecular basis of RSV anti-carcinogenic activities, there have been very few clinical studies commensurate with preclinical findings. Clinical trials in humans are therefore required, in addition to the *in vitro* and *in vivo* animal experiments. At present, RSV is undergoing various phase-I and phase-II interventional trials (US National Institute of Health Clinical Trials website: *www.clinicaltrials.gov*) [11,12]. It is expected that these studies will address the issue of extrapolation from the results of RSV in animal studies to therapeutic potential for humans, and that they will provide a basis for the prospective application of RSV in cancer chemoprevention. It remains to be determined whether the results of randomized clinical trials will corroborate the hypothesis that supplementation with RSV (in combination with radiation, cytotoxic chemotherapy and other targeted molecular compounds) has chemopreventive and/or chemotherapeutic efficacy against malignant gliomas and other human cancers.

Results from the most recent studies performed in rat and human glioma cell lines suggest that the use of RSV in combination with other bioactive food components, such as quercetin and sulforaphane, might be a viable approach for the treatment of human glioma [44,45]. As the biological properties of RSV depend on its bioavailability, it is essential to study this issue before further discussing its chemopreventive efficacy. On account of the fact that pharmacokinetic data from studies with murine models suggest poor bioavailability of RSV, for a further potential application of RSV, isolated or combined, as a cancer chemopreventive agent or adjuvant in chemotherapy, more studies focusing on RSV bioavailability, the half-life of RSV and other bioactive dietary molecules, and the interaction of RSV with other

compounds and their metabolites, as well as studies aimed at devising an appropriate formulation or developing more bioavailable analogues of RSV, are required.

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