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Review Article

Targeting Cancer *Via* Resveratrol-Loaded Nanoparticles Administration: Focusing on *In Vivo* Evidence

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Abstract. Resveratrol (RSV) is a polyphenol endowed with potential therapeutic effects in chronic diseases, particularly in cancer, the second leading cause of death worldwide in the twenty-first century. The advent of nanotechnology application in the field of drug delivery allows to overcome the constrains associated with the conventional anticancer treatments, in particular chemotherapy, reducing its adverse side effects, off target risks and surpassing cancer multidrug chemoresistance. Moreover, the use of nanotechnology-based carriers in the delivery of plant-derived anticancer agents, such as RSV, has already demonstrated to surpass the poor water solubility, instability and reduced bioavailability associated with phytochemicals, improving their therapeutic activity, thus prompting pharmaceutical developments. This review highlights the *in vivo* anticancer potential of RSV achieved by nanotherapeutic approaches. First, RSV physicochemical, stability and pharmacokinetic features are described. Thereupon, the chemotherapeutic and chemopreventive properties of RSV are underlined, emphasizing the RSV numerous cancer molecular targets. Lastly, a comprehensive analysis of the RSV-loaded nanoparticles (RSV-NPs) developed and administered in different *in vivo* cancer models to date is presented. Nanoparticles (NPs) have shown to improve RSV solubility, stability, pharmacokinetics and biodistribution in cancer tissues, enhancing markedly its *in vivo* anticancer activity. RSV-NPs are, thus, considered a potential nanomedicine-based strategy to fight cancer; however, further studies are still necessary to allow RSV-NP clinical translation.

KEY WORDS: anticancer activity; *in vivo* administration; molecular targets; nanoparticles; resveratrol.

INTRODUCTION

Recently, natural compounds with plant origin, the so-called phytochemicals, have attracted the attention of pharmaceutical and medical researchers across the globe due to their comprehensive bioactive properties. Currently, several phytochemicals are being tested in the treatment of numerous diseases. Polyphenols are an example of a group of plant secondary metabolites intensively studied for disease preventing and treatment purposes, primarily on account of their antioxidant properties (1). Particular emphasis is cast upon stilbenes, a subclass of polyphenols. Stilbenes are present in the composition of a wide range of dietary sources, essentially in fruits (e.g., grapes and berries) (2). Chemically, stilbenes are characterized by a C6-C2-C6 basic skeleton, which consists of two phenyl groups linked by an ethene double bond. The most researched compound belonging to stilbenes is resveratrol (RSV), with ca. 10,750 publications currently referenced in the Unites States National Library of Medicine's PubMed service. The grape antioxidant RSV has been attracting great attention due to its recognized health-promoting effects. So far, a large number of studies have been carried out, highlighting its bioactive properties (e.g., antioxidant, anti-inflammatory and cardioprotective) (3), with particular emphasis on the potential anticancer activity of RSV (4–6). In fact, several observations associate the low incidence rates of certain cancer types with the polyphenolic-rich dietary habits of local populations in specific countries (1). In that sense, presently, we are assisting to a rise of the scientific research aiming the clinical application of polyphenols, notably RSV, as a therapeutic molecule for cancer

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treatment, one of the most prominent diseases of the twenty-first century.

Currently, according to the last records of the World Health Organization (WHO), cancer is the second leading cause of death worldwide. In general, cancer is developed through a series of various genetic and epigenetic alterations that promote suppression of tumor suppressor genes and/or induction of a toxic gain of function of key regulatory genes (known as oncogenes) involved in pathways related to cell proliferation, migration, and invasiveness, conducting to an uncontrolled cell growth (7–9). Consequently, all these genetic and epigenetic alterations create the ideal microenvironment to tumor expansion and subsequent invasion and metastasis due to angiogenesis. Bearing this in mind, hyperactivated oncogenes and consequent aberrant protein expression, the silencing of tumor suppressor genes, and consequent absence of protein expression, as well as other tumor microenvironment features, can be considered cancer biomarkers. To rationalize the complex biology of cancer, distinct biological capabilities acquired during carcinogenesis, known as "hallmarks of cancer", were proposed by Hanahan and Weinberg (10) in 2000 and updated by the authors in 2011 (10,11). These cancer hallmarks are key characteristics of cancer biology that hold a double meaning, i.e., they are the cause of the low success rates observed in conventional cancer therapies, but they also contribute to develop more accurate diagnosis techniques and more specific and efficient therapeutic alternatives (12,13). Nonetheless, despite the advances in medicine and technology, the available therapeutic options for cancer are still very invasive to the patient. In fact, chemotherapy is frequently associated with the presence of unspecific and adverse side effects and off-target risks (14–16). Additionally, in the majority of the cases, chemotherapy induces cancer multidrug resistance, compromising the therapeutic efficacy of conventional administered chemotherapeutic agents, as well as the patient well-being.

Thus, in the last few years, nanotechnology emerged as a promising technological strategy to surpass the disadvantages associated with the conventional anticancer treatments, particularly regarding chemotherapy (17–20). This updated concept is based on the use of nanotechnology-based carriers, also known as nanocarriers, to achieve a targeted and controlled drug delivery. Overall, polymeric and lipid-based nanoparticles (NPs) are among the most used nanocarriers for anticancer drug delivery, usually presenting a particle size range between 1 and 1000 nm, being the ideal particle size for this purpose located between 10 and 200 nm (21–23). Typically, NPs are divided into two major groups according to their nature: (i) inorganic NPs, based on carbon, metals and silica (24–27); and (ii) organic NPs, based on lipids (liposomes, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs)), polymers and proteins (28,29). In the development of nanotherapeutic approaches for cancer, the physicochemical properties of NPs (particle size, zeta potential (ZP), and architecture) should be taken into consideration, since these parameters influence the encapsulation efficiency (EE), and the target and successfully controlled drug delivery to cancer cells (22,23,30). In order to ensure a higher therapeutic effect and achieve an upscaling drug accumulation into the tumor site, NPs can be further modulated with molecules (commonly known as ligands) that are specifically recognized by biomarkers (e.g., overexpressed protein receptors) on the surface of cancer cells (23,31–34). Antibodies, proteins, peptides and small molecules are some examples of ligands. Targeting mechanisms allow NPs uptake by active or passive transport, where the latter promotes an enhanced permeability and retention (EPR) effect (17,35,36). EPR effect is associated with an abnormal increase of blood vessels in the tumor, leading to a great extravasation of molecules. However, in tumor cells, the drainage is less efficient, resulting in the accumulation of therapeutic molecules in the target tumor tissue, which is beneficiated by targeted nanodelivery. All the advantages mentioned clearly present NPs as a promising tool to be applied in diagnosis and therapy approaches for cancer, both as an isolated and as in a theranostics perspective (34).

Generally, the use of nanocarriers in drug delivery enhances the drug solubility, biodistribution and bioavailability, ultimately improving the therapeutic effect while using lower drug doses. Thus, nanocarriers constitute a valuable approach to improve the effectiveness of conventional anticancer agents and novel plant-derived anticancer agents, in particular poorly soluble polyphenols that, in their free form, demand the administration of high concentrations to induce an *in vivo* therapeutic activity (21,24,37). Together with their low water solubility, polyphenols, such as RSV and epigallocatechin-3-gallate (EGCG), suffer an extensive metabolization and, thus, exhibit low bioavailability, preventing their delivery in optimal concentrations at the target tissue. In that sense, the encapsulation or conjugation with nanosized structures allows to protect and maintain the structural integrity of polyphenolic compounds, as well as to increase the water solubility (27,38–40). In addition to the physicochemical properties' upgrade, nanotechnology-based carriers convey polyphenols towards the target cells, enhancing their bioavailability and, consequently, their bioactivity.

In the present review, the authors' main purpose is to highlight the *in vivo* anticancer potential of RSV encapsulated or conjugated with nanotechnology-based carriers. Initially, a thorough overview is presented to the reader, starting by describing the inherent RSV physicochemical characteristics, stability and pharmacokinetics. Subsequently, it is presented a brief description of the RSV anticancer molecular targets and its potential as an anticancer agent itself. Finally, this review emphasizes the current advances associated with the design and *in vivo* administration of RSV-loaded nanoparticles (RSV-NPs), as a nanotherapeutic approach towards the treatment of distinct types of cancers.

RESVERATROL

Description

RSV, known as 3,5,4′-trihydroxystilbene (41) or 5-(2-(4-hydroxyphenyl)vinyl)benzene-1,3-diol (42), is classified as a class II compound by the Biopharmaceutical Classification System (BCS) due to its poor water solubility and high membrane permeability (43,44).

From a historical perspective, it was approximately in the year of 1940 that RSV was isolated for the first time from the roots of white hellebore (*Veratrum grandiflorum* O. Loes.) (41,45). It was in the early 1990s that RSV became a

noteworthy molecule due to the association with the "French paradox", which ascribes that the moderate red wine consumption, an alcoholic drink widely consumed in France that exhibits a RSV-rich chemical profile, improves the cardiovascular health of French people despite their high saturated fat diet (45).

Nevertheless, besides present in the skin of red grapes (46), RSV is found in the composition of other edible natural foods in different ranges of concentrations, such as blueberries, raspberries, mulberries, cranberries, peanuts and pistachios (2,41,47). Besides, in nature, RSV appears as a secondary metabolite featured in the following edible and non-edible plant families: *Vitaceae*, *Dipterocarpaceae*, *Gnetaceae*, *Cyperaceae* and *Leguminosae* (47). An important aspect of the RSV molecule is that it is produced in higher plants in response to certain stimuli that include the following: injury, ultraviolet (UV) radiation exposure, ozone exposure, pathogen attack and stress-related responses (e.g., drought), being classified as a natural phytoalexin (48,49).

The extensive list of publications of RSV highlights the wide range of bioactivities potentially enabling the treatment of several human diseases, especially chronic diseases, with higher relevance to cardiovascular diseases and cancer (50,51).

Physicochemical Characteristics

RSV, commercially presented as a solid white powder, evidences the molecular formula $C_{14}H_{12}O_3$, and a molecular weight of 228.247 g mol^{-1} (41). It is registered with the chemical abstracts service registry (CAS) number: 501-36-0 (52). The melting point of RSV is between 253 and 255°C (52). Recently, an additional endothermic peak at 267°C was also associated with the melting of the compound (53).

The chemical structure of RSV comprises two aromatic rings linked by a methylene bridge and three phenolic hydroxyl (–OH) groups (41). The functional groups of RSV (hydroxyl groups, aromatic rings and methylene bond) enable the modification into different active derivates, such as *trans*-3,4′,5-trimethoxystilbene, 4,4′-dihydroxy-*trans*-stilbene, pterostilbene, monoalkoxy, and dialkoxy derivatives, which possess innumerous therapeutic functionalities, enhancing the versatility of RSV.

In terms of solubility, RSV is an hydrophobic compound (1-octanol/water partition coefficient, log $P_{o/w} = 3.1$ (54)) soluble in polar solvents, particularly ethanol at 50 mg ml^{-1} (ca. 200 mM) and dimethyl sulfoxide (DMSO) at 16 mg ml^{-1} (ca. 70 mM) due, mostly, to the three –OH groups of the aromatic rings (52,55). RSV solubility in water is 3 mg 100 ml^{-1} (ca. 0.13 mM), which makes it practically insoluble according to the European Pharmacopeia definition (52).

Pharmacokinetics and Stability Concerns

RSV exists naturally in two geometric isomers, *trans*-RSV and *cis*-RSV with the *trans*-isomer being more abundant and exhibiting superior therapeutic potential, particularly as antioxidant and anticancer agent (43,48,56,57). RSV light exposure (sunlight or artificial UV radiation at 254 or 366 nm) triggers rapid isomerization, i.e., conversion of *trans*-RSV to *cis*-RSV and vice versa, rendering them photosensible and instable (Fig. 1) (43). Nevertheless, *in vitro*, when compared to *cis*-RSV, the *trans*-RSV seems to be more photo- and thermostable (55,57), as well as biologically active (58). The isomerization kinetics of RSV is influenced by several factors, including irradiation time and wavelength; physical state of the molecule (i.e., solid or in solution); temperature; and pH (55,58). In solution, RSV is a highly photosensitive compound which leads to more than 80% of UV-induced isomerization, mainly from *trans*-RSV to *cis*-RSV (48). However, when protected from light, *trans*-RSV is stable for 42 h in neutral aqueous buffers and for a long period (28 days) in acidic pH (pH 1–7), whereas *cis*-RSV is only stable at neutral pH (55,58). In acidic pH, the stability of *trans*-RSV increases, as a result of the protection of the –OH groups by the positively charged hydronium cation (H_3O^+), which prevents the radical oxidation process. In alkaline conditions, *trans*-RSV and *cis*-RSV are not stable due to the deprotonation of the molecule and subsequent auto-oxidation followed by degradation reactions (55). Beyond the pH, the stability of *trans*-RSV in solution is also influenced by the temperature, being higher when stored at lower temperatures. Keeping this in mind, it is important to protect RSV from the light, and reduce the pH of RSV solutions, as well as the storage temperature in order to maintain RSV stability when in solution (58).

In comparison to RSV solutions, RSV solid form evidences higher stability. Despite that, solid RSV tends to remain unstable when exposed to intense and prolonged light and/or high humidity (59). Furthermore, in the presence of oxygen (O_2), RSV tends to be prone to auto-oxidation leading to the production of complex semiquinones and quinines, which are highly toxic to cells (55,57,60). In addition, the auto-oxidation of RSV may give rise to toxic phenantrenoids, such as 2,4,6-trihydroxydihydrophenanthrene (THDHP), impairing the clinical application of the molecule (55).

As regards RSV pharmacokinetics, over the time, it has been assessed in animal models and, more recently, in humans (2,43,61–63). The chemical structure of RSV enables the formation of different molecular complexes which may favor the intestinal absorption and cell permeability when RSV is orally administered (3). Therefore, after oral administration, RSV is directly absorbed by the intestinal epithelium, generally through passive transport or by complexation with integrin proteins. Furthermore, several studies have shown that RSV passes through the intestinal membrane and it is further subjected to a first-pass glucuronidation and sulfate conjugation metabolization, being found in the bloodstream as sulfate and glucuronide derivatives, and in its free form. Following the absorption stage, the remaining RSV is sequestered by the liver and, once more, metabolized into glucuronides and sulfates. After hepatic metabolization, an enterohepatic transport occurs leading to the excretion of RSV conjugates in the bile, being reabsorved in its conjugated forms after being submitted to an enzymatic cleavage by β-glucuronidase enzymes in the small intestine (64–66). RSV can pass the gut without metabolic conversion; however, the occurrence of the hydrogenation of the aliphatic double bound induces its metabolization by β-glucuronidase enzymes present in the intestinal microflora, resulting in dihydroresveratrol (dihRSV) (63,67). This metabolite is excreted in the form of glucuronide and sulfate, but it is also

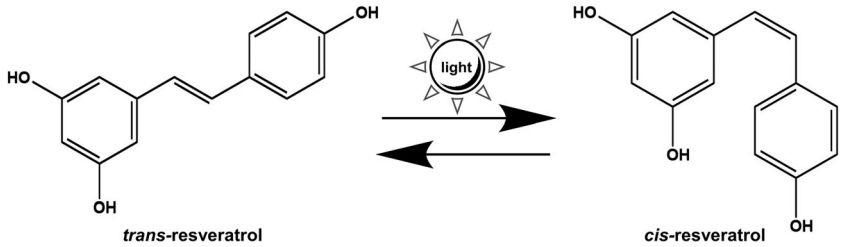

Fig. 1. Resveratrol isomerization triggered by light

absorbed and associated in glucuronide and sulfate conjugates, fact that is in accordance with their presence in plasma and urine (65,67,68).

After the liver stage, the remaining RSV and its metabolites are absorbed into the bloodstream, being transported along with the blood cells, lipoproteins (low-density lipoprotein (LDL) in humans), and plasma proteins, such as albumin, forming complexes that facilitate the uptake of RSV in cells with receptors for these proteins (3,69,70). After oral administration, the plasma half-life of RSV is ca. 8–14 min (71) and the RSV plasma concentrations are generally low, being, in some cases, not measurable (72). Owing to such, even when large doses of RSV (2.5 and 5.0 g) were administered, the obtained blood concentrations did not reach the necessary levels for systemic cancer prevention (66). The most abundant RSV metabolites found in the systemic circulation were sulfates, namely *trans*-RSV-3,5-disulfate and *trans*-RSV-3-sulfate, and glucuronides, precisely *trans*-RSV-3-O-glucuronide and RSV-4'-O-glucuronide (66,69,70,72,73). In plasma, the levels of glucuronides were found to be higher than sulfates, and, in turn, the levels of sulfates are superior than the levels of free RSV (74). In addition, the presence of RSV was verified in various organs, such as in the small intestine, colon, kidney, liver, lungs and brain (75).

Most of the RSV that is given by oral administration is recovered in urine in highly variable amounts (67). In urine, the sulfated forms, notably *cis*-RSV-4′-sulfate (84%), are more abundant than glucuronidated forms (72,76). Free RSV was detected in varying amounts in urine, from only trace amounts up to 17% and 53–85% (66,67,69,77). Additionally, some reports have shown the presence of RSV and its metabolites in human feces, indicating the existence of RSV sulfates in a small quantity (< 1%) (66,67). In sum, the majority of excretion routes of RSV are *via* urine and feces, although the percentage of excreted compounds depends on the experimental conditions used (67).

Regarding the RSV intravenous (i.v.) administration, the apparent terminal elimination half-life ($t_{1/2}$) ranges from 7.8 to 35 min (64,78,79). Das and co-workers focused their attention in the influence of the RSV dose in the RSV pharmacokinetic profile in adult male Sprague-Dawley rats (250–300 g) (80). The findings of Das *et al.* (80) study reported that the area under the concentration-time curve (AUC) is directly proportional to the RSV dose, whereas the clearance is inversely proportional, i.e., high doses of RSV (25 mg kg^{-1}) lead to an increase in the AUC and decrease in clearance. Therefore, the i.v. administration of high doses of RSV may cause a saturation in its elimination and, consequently, increase RSV plasma levels (80). Following RSV i.v. administration, the plasma concentration time profile in animal models normally shows a rapid decline, followed by an increase in RSV concentration that corresponds to the RSV enterohepatic circulation and, finally, after a short time, RSV becomes undetectable (79–81). In Walle *et al.* (67) study, after the i.v. administration of a small dose of ^{14}C-labeled RSV (0.2 mg; 0.8 μmol), the authors concluded that RSV exhibits a high absorption rate, as well as a rapid decrease in plasma concentrations over the first 1 h without any second visible peak. This indicates that the distribution phase occurs rapidly and is extensive. Therefore, presumably, only RSV traces enter the enterohepatic cycle. The authors calculated a $t_{1/2}$ of 9.2 h after oral administration and 11.4 h after i.v. injection (67). The parenteral route is proposed to circumvent concerns associated with the oral administration of RSV (82). However, and similarly to the oral administration of RSV, in the i.v. and intraperitoneal (i.p.) administration, the elimination rate and clearance occur at a fast pace due to the metabolic instability of RSV, i.e., the chemical structure favors the conjugation of RSV with glucuronic and/or sulfonic acid (83). In the study of Zunino and colleagues, after 15 min of RSV i.p. injection (20 mg kg^{-1} body weight), 13 μM of RSV sulfate and 5 μM RSV glucuronide were detected in the mice serum (84). This rapid metabolization impaired the anticancer activity of RSV in a non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice model engrafted with human acute lymphoblastic leukemia (ALL) that present the chromosomal translocation t(4;11) (SEM cell line) (84).

The current weight of evidence regarding RSV pharmacokinetics evidences some discrepancies since it depends, essentially, on the administration route and RSV dose (83). The RSV pharmacokinetic studies contemplate predominantly the oral administration route of RSV, in which, as referred, RSV is quickly absorbed and metabolized. In fact, the gut microbioma role in the metabolization of RSV is noteworthy and requires further investigation regarding its interference when assessing the therapeutic effects of RSV. Facing this scenario, and as will be further discussed, the majority of the targeting-cancer studies administrate RSV *via* i.v. or i.p. injection (85). Nevertheless, the i.p. administration holds limitations, namely the referred premature clearance, setbacks on drug delivery and penetration in the target tissues. Additionally, particularly in cancer therapy, the i.p. administration route is restricted to chemotherapy for cancers with peritoneal involvement (86). Likewise, the i.v. administration allows the distribution of anticancer drugs, such as RSV, *via* the bloodstream throughout the whole organism inducing toxic effects, not only in cancer cells but also, and more rapidly, in normal cells. Additionally, the low water solubility of RSV allied with the inherent instability and rapid metabolism may restrain the i.v. and i.p. administration, since,

in these two types of routes, the drug must be preferably solubilized in aqueous media. This substantiates the urgent unmet need for effective technological strategies to surpass these problems, emphasizing the potential role assumed by the use of RSV-NPs.

RESVERATROL CANCER MOLECULAR TARGETS

RSV is considered an effective anticancer and antiproliferative agent due to its molecular activity, although RSV chemical instability, low water solubility, and bioavailability constitute serious obstacles for its application in the pharmaceutical industry. The first report regarding the anticancer activity of RSV was published in 1997. Jang and colleagues (5) concluded that RSV specifically inhibits the process of oncogenesis in mammary and skin cancer models thanks to its capability of acting on cell pathways involved in the development and progression of cancer. In fact, RSV is involved in the regulation of three key mechanisms, namely apoptosis, proliferation and inflammation, revealing its potential as a chemotherapeutic and chemopreventive agent (4). Thus, RSV acts as a proapoptotic agent. In normal cells, caspase pathways have a key regulatory role in apoptosis induction; however, in cancer cells, the referred mechanism is usually inhibited due to the suppression of key regulatory tumor suppressor genes. RSV proapoptotic effect was studied in a mice model of colon cancer, and the findings suggested that RSV promoted an enhancement of the caspase activity and blocked the expression of antiapoptotic proteins belonging to the heat shock proteins (HSPs) group (87). Another important RSV molecular target is the *TP53* gene, a critical tumor suppressor gene, which has attracted a lot of attention from researchers worldwide (88). The wild-type protein nuclear transcription factor, p53, encoded by the *TP53* gene, blocks tumor development and it is involved in the regulation of different pathways. In cancer, the loss of function of the p53 protein leads to the inhibition of apoptosis and activation of several pathways involved in cancer survival and progression. Several studies reported that, in breast and prostate cancer cells, RSV activates the p53 protein through the interaction with the mitogen-activated protein kinases (MAPK) pathway inducing cell cycle arrest and apoptosis (89,90). Furthermore, RSV induces apoptosis through the interaction with further proteins, as, e.g., the protein kinase Akt, subsequently promoting the activity of forkhead box O3 protein (FOXO3a) through the dephosphorization of this tumor suppressor protein (91). Besides that, it is suggested that RSV, as well as its metabolites, blocks DNA synthesis through cell cycle arrest interfering with cancer cell proliferation (92–94).

The aforementioned effects, essentially the capability of RSV to modulate cell cycle and to promote apoptosis in cancer cells, are strictly linked with RSV chemopreventive activity (56). Noteworthy are the phytochemical simultaneous antioxidant and pro-oxidant properties. The antioxidant effect of RSV is related to the compound's chemopreventive activity, since RSV reduces the impairment of damage caused by biomacromolecules. On the other hand, RSV chemotherapeutic potential is associated with the generation of reactive oxygen species (ROS) in cancer cells which, subsequently, leads to apoptosis. In addition, in normal endothelial cells, high doses of RSV (superior to 50 μM) may revert the protection against spontaneous apoptosis due to the interaction with the NO/cGMP-dependent protein kinase I (cGMP/PKG-I) signaling pathway. RSV downregulates PKG-I, reducing the inhibitor of apoptosis proteins (IAPs), subsequently inducing apoptosis and inhibiting angiogenesis (endothelial cell proliferation). The RSV neo-angiogenesis prevention may occur by the same formerly described mechanism (95).

RSV holds an antiproliferative activity due to its ability to alter the signaling activity, hampering cancer progression. For example, RSV suppresses the tumor growth of myeloma cells through the inhibition of nuclear factor kappa B (NF-κB), a transcription factor that is involved in cancer progression *via* the activation of genes that encode antiapoptotic proteins (96). The NF-κB suppression by RSV caused the downregulation of antiapoptotic proteins: Bcl-2, Bcl-xL, X-linked inhibitor of apoptosis protein (XIAP), anti-cellular inhibitor of apoptosis protein (c-IAP) and vascular endothelial growth factor (VEGF). Specifically, the downregulated expression of Bcl-2, subsequently, leads to the inhibition of cytochrome C release from the mitochondrial intermembrane space, promoting intrinsic cell apoptosis (56,96). Another example is the RSV interaction with the growth factor heregulin-beta 1 (HRG-β1) that activates a cascade of signaling pathways that includes the expression of matrix metalloproteinase (MMP), in particular MMP-9 (end product regulated by NF-κB), which is correlated with an aggressive type of breast cancer (97). RSV targets the HRG-β1 growth factor inhibiting the HRG-β1 pathway and, consequently, MMP-9 expression, thereby promoting an antiproliferative effect in human breast cancer cells.

Cancer and inflammation walk alongside in the early oncogenesis stages and RSV has already proved its anti-inflammatory activity. RSV is a potent anti-inflammatory compound since it inhibits pro-inflammatory mediators, as well as the activity of cyclooxygenase (COX) enzymes (cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2)), through the inhibition of transcription factors, such as NF-κB or activator protein-1 (AP-1) (98). In a recent study, performed in 7,12-dimethylbenz(a)anthracene (DMBA)-induced breast cancer rat models, RSV suppressed COX-2 enzymatic activity (99). Furthermore, it was shown that the antiapoptotic protein Akt upregulates the expression of COX-2; however, RSV interacts with the protein Akt inhibiting its expression, thus, consequently, suppressing the activity of the COX-2 enzyme (100). The inhibition of COX enzymes leads to an overexpression of macrophage inhibitory cytokine-1 (MIC-1), reducing cancer cell proliferation (101). For visual representation of the chemotherapeutic and chemopreventive RSV molecular targets outlined in this section, the reader is referred to Fig. 2.

ANTICANCER ACTIVITY OF RESVERATROL-LOADED NANOPARTICLES

The aforementioned critical characteristics of RSV, including its poor water solubility, its chemical instability allied with its reduced bioavailability and short half-life, lead together to ineffective *in vitro* and, above all, *in vivo* effects, strongly impairing its recognized bioactivity (50,57). So far,

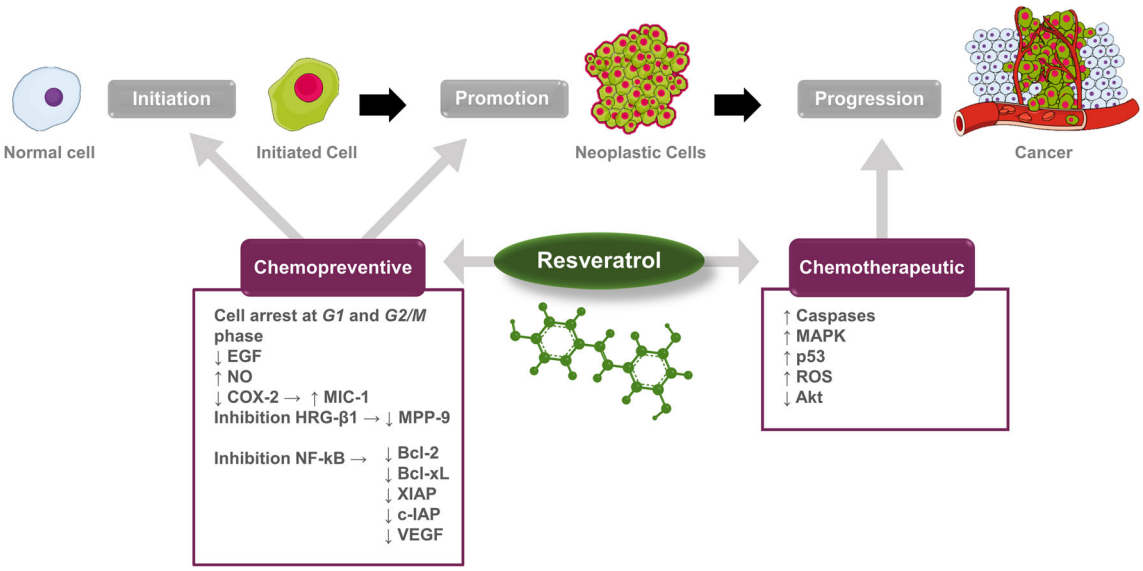

Fig. 2. Chemotherapeutic and chemopreventive resveratrol molecular targets during oncogenesis. c-IAP anti-cellular inhibitor of apoptosis protein; COX-2 cyclooxygenase-2; EGF epidermal growth factor; HRG-β1 heregulin-beta 1; MAPK mitogen-activated protein kinases; MIC-1 macrophage inhibitory cytokine-1; MMP-9 matrix metalloproteinase-9; NF-κB nuclear factor kappa; NO nitric oxide; ROS reactive oxygen species; VEGF vascular endothelial growth factor; XIAP X-linked inhibitor of apoptosis protein

several studies have proposed that the incorporation of RSV in nanocarriers, particularly NPs, may surpass these limitations by improving the solubility, stability, and bioavailabity of RSV, enabling an effective therapeutic action in cancer *in vitro* and *in vivo* models (Fig. 3).

In this section, we will focus on studies concerning the anticancer activity of RSV-NPs in *in vivo* models tested so far, including colorectal, brain, ovarian, pancreatic and lung cancers. A description of the RSV-NPs production methods used, their main physicochemical features, as well as the *in vitro* cytotoxicity, biodistribution, and *in vivo* effects of RSV on the tumor growth in cancer animal models will be presented hereinafter. Attending to the existence of *in vivo* evidence only performed in normal and cancer models, Table I summarizes the biodistribution effects of different types of RSV-NPs. Table II summarizes, according to the referred cancer types, the *in vivo* anticancer effects observed after the administration of different RSV-NPs.

Colorectal Cancer

Colorectal cancer is the third most common type of cancer worldwide. It is a primary neoplastic disease whose genesis occurs in the large intestine (110). The available chemoterapy induces adverse side effects and, due to extended treatment, the colorectal cancer cells may gain a resistant phenotype, encouraging the development of new therapeutic strategies. In this context, it is proved in the literature that RSV inhibits the invasion and metastasis of colorectal cancer cells (111).

This way, in both *in vitro* and *in vivo* colorectal cancer models, polyethylene glycol-polylactic acid (PEG-PLA) polymeric NPs were tested as potential RSV nanocarriers (106). The solvent-evaporation method was used in the development of RSV-loaded PEG-PLA-NPs (RSV-PEG-PLA-NPs). The obtained RSV-PEG-PLA-NPs evidenced a mean particle size of 119.9 nm, a ZP of −4.6 mV, and an EE of 52.9%. Moreover, the evaluation of the kinetic release pattern demonstrated a prolonged RSV release profile from the NPs. In the same study, it was observed that the RSV-PEG-PLA-NPs reduced the viability of murine colon carcinoma cells (CT26 cell line) in a dose-dependent manner, exhibiting a half maximal inhibitory concentration (IC50) of 16.0 mM. In comparison with free RSV, the RSV-PEG-PLA-NPs displayed a higher effectiveness on the triggering the apoptotic pathways of CT26 cells, allowing a greater release of caspase-3 and cleavage of poly (ADP-ribose) polymerase (PARP) (106). It is known that RSV, in cancer cells, suppresses the glucose uptake through the inhibition of intracellular ROS, with subsequent downregulation of hypoxia-inducible factor-1α (HIF-1α) (112). The loading of RSV into PEG-PLA-NPs increased the suppression of glucose uptake comparatively to free RSV in colon cancer cells (106). The effect of RSV-PEG-PLA-NPs on the ^{18}F FDG uptake was tested on BALB/c nude mice submitted to a subcutaneous injection of CT26 colon cancer cells. The mice control group that was intravenously administered with empty PEG-PLA-NPs showed a significant increase in the tumor-to-background (T/B) ratio to 168.2%, whereas the group administered with RSV-PEG-PLA-NPs displayed a decrease in tumor ^{18}F FDG uptake, reducing the T/B uptake to 62.0%. In fact, the treatment with RSV-PEG-PLA-NPs led to a significantly decrease in tumor growth in mice. On the other hand, treatment with empty PEG-PLA-NPs presented a sharp increase in tumor growth with a survival rate of 11.4 days for the control and 18 days for the RSV-PEG-PLA-NP mice group. This experiment revealed that polymeric NPs, particularly those based on PEG and PLA, are a suitable approach to load RSV for anticancer purposes. These polymeric NPs enhanced the stability and circulation time of RSV, promoting metabolic and not only anticancer effects *in vitro*, but also *in vivo*, in murine colon carcinoma models (106).

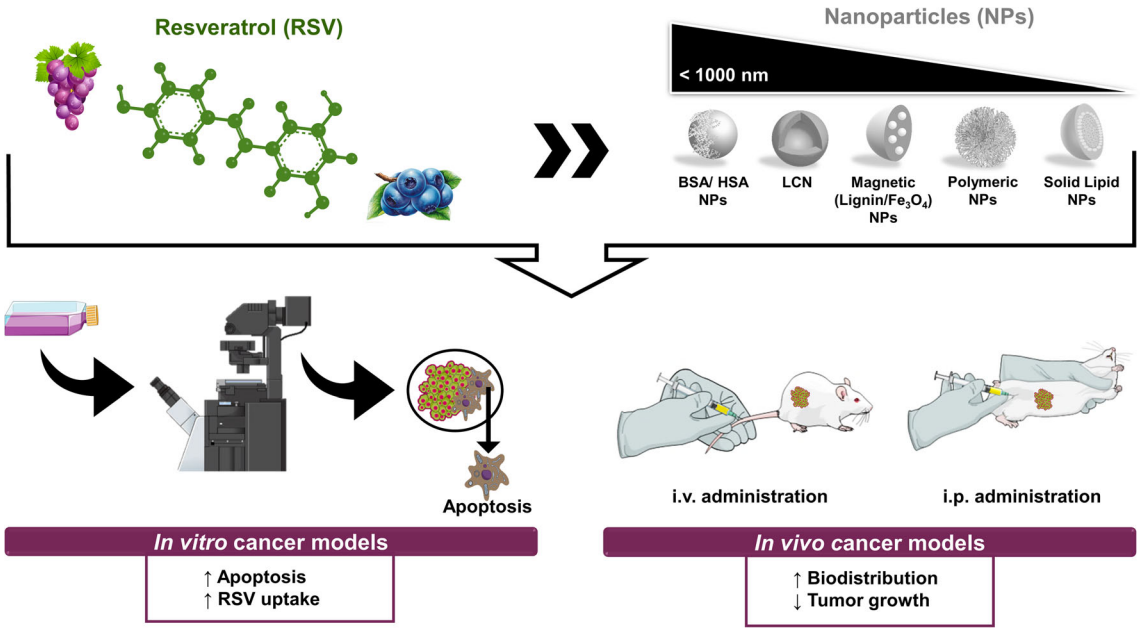

Fig. 3. Schematic representation of the anticancer activity demonstrated by resveratrol nanotherapeutic approaches after *in vitro* testing in cancer cell lines, and *in vivo*, particularly after intravenous (i.v.) and intraperitoneal (i.p.) administration in cancer-bearing animal models. BSA bovine serum albumin; HSA human serum albumin; LCN lipid-core nanocapsules

Brain Cancer

Gliomas are the most malignant primary tumors of the brain, with a short-term clinical prognosis both in adults and children (113). The scarcity of an effective therapy for such type of tumor is intimately related to their resistance to conventional treatments. The blood-brain barrier (BBB) is formed by highly specialized endothelial cells that contain specific cellular transporters, ATP-binding cassette (ABC) transporters, which actively prevent the passage of xenobiotics to the central nervous system (CNS) (114). Nevertheless, the natural protection conferred by the BBB constitutes a hindrance to the delivery of drugs intended to treat CNS pathological conditions, such as brain cancer. In gliomas, the BBB function is compromised due to several factors related to the tumor microenvironment or with the tumor ability to upregulate the expression of export transporters forming a blood-tumor barrier (BTB).

In a recent work, it was demonstrated that RSV is able to suppress the expression of matrix metalloproteinase-2 (MMP-2) and activate the RhoA/Rho-associated kinase (ROCK) signaling pathway inhibiting glioblastoma cell motility and invasiveness (115). Nevertheless, the instability and pharmacokinetic limitations of RSV allied with the restrictions of the BBB penetration and BTB calls for the development of RSV-NPs to selectively target the tumor site. Facing this purpose, recently, researchers have developed transferrin (Tf)-modified PEG-PLA-NPs conjugated with RSV (Tf-NPs-RSV) with a particle size of 153.3 nm to target gliomas (107,116). Tf, which regulates the cellular iron uptake, was used as a ligand

Table I. Examples of the RSV Biodistribution Effects Displayed by Distinct Types of RSV-NPs in Normal and Cancer Animal Models

	Tissue	Type of RSV-NPs	RSV dose (mg kg^{-1})	Animal species	Sex	Age	Weight (g)	*Via*	Time after administration	RSV biodistribution effects*	Ref.
Normal animal models	Brain	SLNs	5	Wistar rats	Female	Adult	150–200	i.p.	90 min	6.0-fold higher	(102)
	Liver, kidney, heart, and ovaries	BSA	1.5	Kunming mice	Female	n/a	18–20	i.v.	n/a	2.1-fold higher	(103)
Cancer animal models	Pancreatic cancer	HSA	n/a	Balb/c nude mice	n/a	4 – 5 weeks	n/a	i.v.	24 h	8.1-fold higher	(104)
	Lung cancer	Magnetic (lignin/Fe_3O_4)	10	C57BL/6 mice	Female	6 – 7 weeks	n/a	i.v.	24 h	6.7-fold higher	(105)

n/a not available, *RSV* resveratrol, *RSV-NP* resveratrol-loaded nanoparticle, *i.p.* intraperitoneal, *i.v.* intravenous, *SLN* solid lipid nanoparticle, *BSA* bovine serum albumin, *HSA* human serum albumin
*In comparison with the administration of free RSV

Table II. Overview of the *In Vivo* Anticancer Effects of Different Types of RSV-NPs According to Cancer Types

Cancer type	Cancer model	Type of RSV-NPs	RSV dose (mg kg^{-1})	Animal species	Sex	Age	Weight (g)	*Via*	Treatment duration	Anticancer effects			Ref.
										Tumor volume reduction	Tumor growth inhibition	Additional effects	
Colorectal	CT26 murine colorectal cancer cells	Polymeric (PEG-PLA)	100 (twice *per* week)	Balb/c nude mice	n/a	n/a	n/a	i.v.	3 weeks	ca. 2.6-fold*[1]	66.9% (RSV-NPs)	18 days of extended survival (for RSV-NPs)	(106)
Brain	C6 rat glioma cancer cells	Polymeric (PEG-PLA)	15 (every 2 days)	Sprague-Dawley rats	Male	n/a	200–250	i.p.	n/a	n/a	ca. 2.6-fold increase*[2]	69.8% of extended survival*[3]	(107)
	U87 human glioblastoma cells	Polymeric (mPEG-PCL)	10	Nude mice	n/a	6–8 weeks	18–20	i.p.	n/a	ca. 1.6-fold*[4]	n/a	Increased anticancer efficacy	(108)
	C6 rat glioma cells	LCN	5 (once *per* day)	Wistar rats	Male	8–9 weeks	250–350	i.p.	10 days	ca. 4.3-fold*[5]	n/a	n/a	(109)
Ovarian	SKOV3 human ovarian cancer cells	BSA	200, 100, and 50	Balb/c (nu/nu) nude mice	Female	4 weeks	18–20	i.p.	4 weeks	ca. 1.5-fold*[6]	ca. 1.3-fold increase*[7]	ca. 1.1-fold reduction*[8]	(103)
Pancreatic	PANC-1 human pancreatic cancer cells	HSA	10	Balb/c nude mice	n/a	4–5 weeks	n/a	i.v.	35 days	ca. 15.0-fold*[9]	n/a	n/a	(104)
Lung	LLC murine Lewis lung carcinoma cells	Magnetic (lignin/Fe_3O_4)	10	C57BL/6 mice	Female	6–7 weeks	n/a	i.v.	n/a	ca. 5.2-fold*[10]	ca. 3.9-fold increase*[11]	n/a	(105)

n/a not available, *RSV* resveratrol, *i.v.* intravenous, *i.p.* intraperitoneal, *RSV-NP* resveratrol-loaded nanoparticle, *PEG* polyethylene glycol, *PLA* polylactic, *mPEG* methoxy polyethylene glycol, *PCL* poly-ε-caprolactone, *Tem* temozolomide, *LCN* lipid-core nanocapsule, *BSA* bovine serum albumin, *HSA* human serum albumin

*[1] Empty NPs/RSV-NPs (tumor volume ratio, %)

*[2] RSV-NPs/free RSV (tumor volume ratio, %)

*[3] In comparison with the administration of free RSV

*[4] Free Tem + free RSV/RSV-NPs (relative tumor volume ratio, %)

*[5] Free RSV/RSV-NPs (tumor volume ratio, mm^3)

*[6] Free RSV/RSV-NPs (tumor volume ratio, mm^3)

*[7] RSV-NPs/free RSV (tumor growth inhibition rate ratio, %)

*[8] Free RSV/RSV-NPs (tumor weight ratio—considering the higher dose, g)

*[9] Free RSV/RSV-NPs (relative tumor volume ratio, %)

*[10] Free RSV/RSV-NPs (tumor volume ratio, mm^3)

*[11] RSV-NPs/free RSV (tumor growth inhibition rate ratio, %)

to improve the NPs specificity to bind the target glioma cells, since Tf receptors are remarkably expressed in gliomas (117). Tf-NPs-RSV were prepared using the double emulsion and solvent evaporation method followed by incubation with purified Tf and addition of RSV (107,116). The conjugation of RSV with the Tf-modified PEG-PLA-NPs significantly improved the dissolution properties of RSV. In the cytotoxicity studies, Tf-NPs-RSV decreased the viability of rat C6 glioma cells and human U87 glioma cells mainly due to the high uptake of the NPs in comparison with free RSV. The conjugation of RSV with Tf-modified PEG-PLA-NPs significantly enhanced the apoptosis of the glioma cells (C6 and U87). The antitumor activity of the Tf-NPs-RSV was, then, evaluated in a glioma-bearing rat model: C6 glioma-bearing rats. Animals were injected intraperitoneally with the same dose (15 mg kg^{-1}) of Tf-NPs-RSV, NPs-RSV, and free RSV. Tf-NPs-RSV presented the highest survival rates, with an increase in life span of 69.8% over the free RSV group. These results sustain that Tf-modified PEG-PLA-NPs increase RSV therapeutic effects in the presence of lower doses by overcoming RSV low bioavailability and directing the drug delivery to the target tissue, consequently promoting an accumulation of a higher concentration of RSV in the tumor site, as desired (107).

RSV was used as a chemosensitizer in order to enhance the anti-glioma effect of temozolomide (Tem) and reduce its toxicity (108). A nanoformulation co-loading RSV and Tem was developed by nanoprecipitation. The methoxy polyethylene glycol-poly-ε-caprolactone (mPEG-PCL) was used as a copolymer. Produced NPs showed a particle size of 135.3 nm and a ZP of − 3.4 mV. Drug loading (DL) was 12.4% for RSV and 9.3% for Tem. In addition, the EE values of Tem and RSV were superior to 80.0%. In the *in vitro* studies, Tem/RSV-co-loaded mPEG-PCL-NPs significantly inhibited the expression of Akt proteins in glioma cells preventing the activation of the phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) signaling pathways, which are directly related to survival and proliferation of cancer cells. In addition, in glioma cells, Tem/RSV-co-loaded mPEG-PCL-NPs promoted the expression of proapoptotic proteins (108,118). In the same study, the *in vivo* antitumor effects of the Tem/RSV-co-loaded mPEG-PCL NPs were evaluated in a U87-xenograft nude mice model. A significant superior tumor growth delaying effect was observed when Tem/RSV-co-loaded mPEG-PCL NPs were administrated to the mice relatively to the results observed in mice treated with the single combination of free drugs. Therefore, Xu *et al.* (108) proved that RSV can act as an adjuvant, increasing synergistically the chemotherapeutic effect of Tem when co-administered in polymeric NPs in U87 human glioma cells and in a xenograft glioma mice model (108).

Nanocapsules are polymeric NPs with a core-shell structure attained by the interfacial deposition of polymers (109,116). The hydrophobic nature of these polymeric nanocapsules enables the encapsulation of hydrophobic drugs, such as RSV. The effects of the encapsulation of RSV into lipid-core nanocapsules (LNCs) were tested in *in vitro* and *in vivo* models of glioma (109). The LNC nanosuspensions were prepared by interfacial deposition of poly-ε-caprolactone. The obtained RSV-LNCs evidenced a particle size of 253.0 nm, a ZP of − 13.5 mV at a pH of 4.71 and a RSV content of 0.935 mg mL^{-1}. In *in vitro* tests, C6 glioma cell viability after exposure to RSV-LNCs suffered a significant reduction thanks to the capacity of RSV-LNCs to induce cell cycle arrest and apoptosis. The *in vivo* tests were performed in rats bearing C6 glioma implants. A great tumor size reduction was observed after i.p. administration of 5 mg kg^{-1} day^{-1} of RSV-LNCs for 10 days (ca. 34.26 mm^3), confirming the enhanced anticancer potential of RSV after nanoencapsulation. Furthermore, in the rat model, the administration of RSV-LNCs decreased some commonly observed glioma histopathological features, such as intratumoral hemorrhaging, peritumoral edema and peripheric pseudopalisading. All these findings suggest that LNCs improve RSV delivery across BBB, being able to target brain cancers and, with a reduced RSV concentration, an effective anticancer bioactivity may be achieved (109).

SLNs loaded with RSV were produced as a different approach to target glioma cancer (102). SLNs show a biocompatible and biodegradable nature as well as the ability to bypass the BBB, being less toxic when compared to polymeric NPs. SLNs are solid colloidal systems at room and body temperature. SLNs have an inner solid lipid matrix and an outer shell where the surfactant is adsorbed to inhibit the aggregation of the dispersed SLNs. To target glioma cells, 12 formulations of RSV-loaded SLNs were produced by solvent evaporation technique employing high-speed homogenization followed by ultrasonication. Glyceryl behenate (Compritol 888 ATO®) was used as lipid and the optimal RSV:lipid ratio was 1:10. The optimal formulation was developed using a combination of surfactants, Tween 80 and polyvinyl alcohol (PVA) presenting a small particle size of 248.3 nm and a ZP of − 25.5 mV. The EE of the optimal formulation was 33.9% and the DL was 3.1%. The optimal RSV-loaded SLNs exhibited a typical biphasic *in vitro* release profile and the kinetics of this formulation was fitted to the Higuchi model, presenting a drug release pattern controlled by diffusion. Cytotoxic studies performed in rat C6 glioma cell line confirmed the innocuity of the SLNs and enabled to ascertain that the anticancer activity of RSV remains after the encapsulation process. *In vivo* studies were performed in Wistar rats and demonstrated that RSV-loaded SLNs evidenced a higher RSV accumulation in the brain when compared to free RSV (ca. 17.28 μg g^{-1} *vs* ca. 3.45 μg g^{-1}, respectively). This difference in the RSV accumulation was possibly due to the presence of Tween 80 that provides a hydrophilic layer around SLNs, reducing its uptake to other organs (spleen, lungs, kidney and liver) and enhancing the uptake by BBB cells. Hence, SLNs constitute a cost-effective strategy to improve the biodistribution of RSV in the brain, enabling the use of lower RSV concentrations and, subsequently, contributing to the reduction of possible adverse side effects (102).

Ovarian Cancer

The ineffective therapeutic action of the currently used chemotherapeutic agents combined with ovarian cancer increasing incidence and low 5-year survival rate prompt the search of new anti-ovarian cancer drugs (103). RSV is a potential therapeutic agent for the treatment of ovarian

cancer since it inhibits the proliferation, migration, and invasion of ovarian cancer cells (119). Furthermore, in a SKOV3 cell-xenograft BALB/c nude mice model, the i.p. administration of a RSV solution (RSV dissolved in dehydrated alcohol and diluted in PBS, 100 mg kg^{-1}) reduces ovarian cancer growth (119). The activity of RSV-bovine serum albumin NPs (RSV-BSA-NPs) against ovarian cancer was studied in nude mice. The BSA-NPs were used as a strategy to overcome the low solubility of RSV and improve its activity *in vivo* (103). The formulation of RSV-BSA-NPs was first developed in a previous study (120). The RSV-BSA-NPs were produced by a desolvation method resulting in a particle size between 400 and 500 nm. The content of RSV in the BSA-NPs was 4.077 mg, the EE was 33.9%, and results suggested a water solubility improvement. In SKOV3 cells, RSV-BSA-NPs inhibited cell proliferation in a dose-dependent manner, inducing a significant proapoptotic effect when compared with the exposure to free RSV (120). SKOV3 cells were implanted subcutaneously in Balb/c nude mice (103). Subsequently, the tissue distribution of RSV in a tumor-bearing mice model was evaluated and it was proved that RSV-BSA-NPs administrated by i.v., through the tail vein, improved the concentration of RSV in the liver, kidney, heart and ovaries (with a targeting efficiency varying from 27.13 to 57.47%), while the detected concentration in blood was much lower. Additionally, 42 and 49 days after the i.p. administration of RSV-BSA-NPs, the tumor growth was markedly retarded and the inhibition rate was higher than in mice treated only with RSV. A terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and a Western blot analysis proved that the tumor growth inhibition in the ovarian cancer mice model was correlated with the activation of the mitochondrial apoptotic pathway. Thus, RSV-BSA-NPs represent a different and promising anticancer therapeutic approach, even though further studies related to the underlying molecular mechanisms of the RSV-BSA-NPs anticancer activity are required (103).

Pancreatic Cancer

Human serum albumin (HSA) is an endogenous protein that presents non-toxicity, non-immunogenicity and a good biocompatibility, gathering promising characteristics for a candidate material for the development of nanocarriers for low soluble compounds, as RSV (104). The presence of functional groups in its structure allows the functionalization with different ligands. Thus, in Geng *et al*. (104) study, RSV-loaded HSA NPs conjugated with arginine-glycine-aspartate (RGD) *via* a PEG "bridge" (HRP-RGD NPs) were produced using a simple desolvation method. The HRP-RGD-NPs were produced to target pancreatic cancer cells and exhibited an average particle size of 120.0 nm with a spherical-shaped morphology, in which the presence of RSV was confirmed by UV spectroscopy. The EE value for RSV was 62.5% and the release rate was 58.4% after 60 h and pH 5, the highest RSV release value compared to the values observed for pH 6.5, pH 7.4, and pH 9.0 at 37 °C. These data suggested that the use of HSA, as an RSV nanocarrier, increases the RSV release at lower pH values indicating that HRP-RGD NPs may potentially enhance the RSV anticancer activity, since cancer cells hold a lower pH in comparison with normal cells. The exposure of pancreatic cancer cells (PANC-1 cell line) to HRP-RGD NPs triggered the appearance of pyknotic nuclei, a clear sign of apoptosis. Subsequently, in PANC-1-xenografted mice, after i.v. injection of HRP-RGD NPs, an improvement of 5.43-fold was observed in the blood circulation time when compared to free RSV. In the tumor-bearing mice, the HRP-RGD NPs increased RSV accumulation on the target site, probably due to the targeting effect of RGD and the improved EPR effect attributed to HSA and PEG (Fig. 4). The EPR effect allows drugs to accumulate in tumors by escaping the reticuloendothelial system (RES) increasing, consequently, the accumulation and retention of the drug in tumors, which leads to an enhancement of the treatment efficacy while decreasing the side effects (21). In the tumor-bearing mice used in Geng *et al.* (104) research, after 35 days of treatment with HRP-RGD NPs, the tumor growth was actively suppressed, contrarily to the results observed in the groups administered with PEG-coated RSV-loaded HSA NPs without RGD (HRP NPs) and free RSV, in which the tumor kept growing (Fig. 4). No toxicity was found in the major organs granting the safety features of the produced HRP-RGD NPs and suggesting their use as a great approach to target pancreatic cancer (104).

Lung Cancer

RSV has proven to be a promising and viable potential therapeutic agent to target lung cancer cells (121). In this regard, it has been used in nanomedicine approaches to treat lung cancer.

Lignin is an aromatic organic macromolecule, derived from the cell wall of vascular plants, with good biocompatibility, and pH and thermal stabilities (105). Therefore, combined with its non-toxic nature, lignin has proved to be an excellent polymeric drug carrier to enhance RSV availability and bioactivity in lung cancer cellular and mice models. Alkali lignin (AL) was used to prepare RSV-loaded NPs by a self-assembly method, together with iron oxide (Fe_3O_4), which was used to provide magnetic properties to the NPs. An AL concentration of 0.5 mg mL^{-1} in methanol with a final water content of 90% was chosen to achieve uniform spherical hollow NPs. In these conditions, the magnetic RSV-loaded lignin NPs (AL/RSV/Fe_3O_4-NPs) presented a particle size of ca. 160.0 nm, which was in accordance with the established particle size values to be delivered and accumulated in tumors (between 100 and 200 nm). The use of Fe_3O_4 in the assembly of the NPs was successful, allowing to reach a magnetic saturation value of 24.6 emu g^{-1}. The magnetic properties of AL/RSV/Fe_3O_4-NPs demonstrated to modulate RSV release. The AL/RSV/Fe_3O_4-NPs, under magnetic field, evidenced a prolonged RSV release. Furthermore, the AL/RSV/Fe_3O_4-NPs improved RSV structural stability (only 2% of *trans*-RSV suffered isomerization after 1 h of direct sunlight). The safety of AL/RSV/Fe_3O_4-NPs was proved using an hemolysis test, which demonstrated a reduced hemoglobin release from red blood cells (< 5%), contrary to the commercial formulations of RSV that possess harmful excipients in their composition. The assembly of AL/RSV/Fe_3O_4-NPs increased the RSV efficacy, probably due to the slow RSV release that increases the chemosensitivity of lung cancer cells and, subsequently,

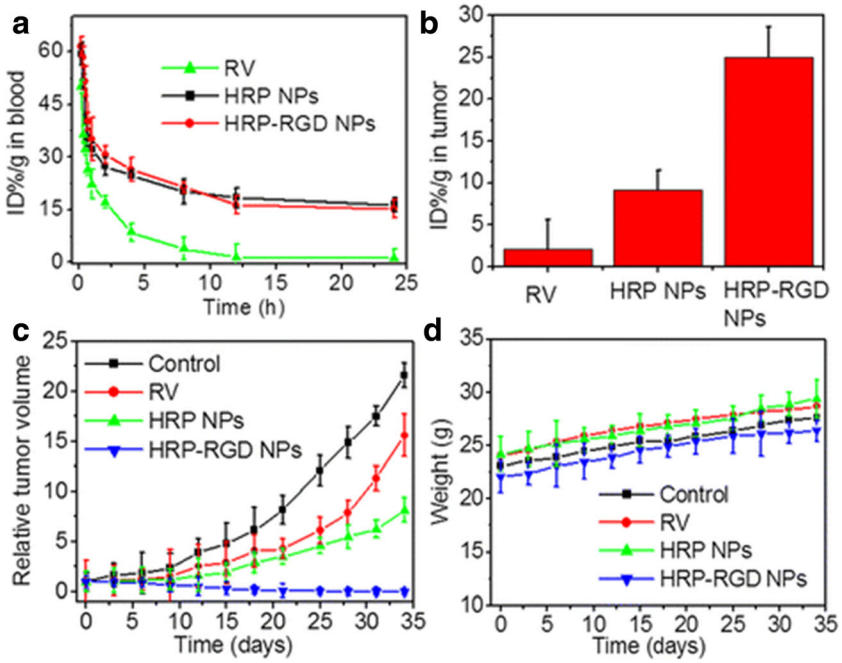

Fig. 4. **a** Blood circulation curves of free RV, PEG-coated RV-loaded HSA nanoparticles (HRP NPs), and resveratrol (RV)-loaded HSA nanoparticles conjugated with RGD (arginine-glycine-aspartate) *via* PEG (HRP-RGD NPs) in mice after intravenous injection determined by the RV absorbance from diluted tissue lysate. **b** Content of RV in tumor at 24 h post-treatment with RV, HRP NPs, and HRP-RGD NPs. **c** The relative tumor volume of tumor-bearing mice after intravenous injection with saline (control), RV, HRP NPs, and HRP-RGD NPs. **d** Body weight of tumor-bearing mice after intravenous injection with saline (control), RV, HRP NPs, and HRP-RGD NPs. Reprinted with permission from (104). Copyright (2019) Springer. HSA human serum albumin; ID%/g percentage of injected dose per gram of tissue; PEG polyethylene glycol; RV resveratrol

enables RSV internalization, ultimately reducing the toxicity in normal tissues. Additionally, in Dai *et al.* (105) research, murine Lewis lung carcinoma (LLC) tumor-bearing C57BL/6 mice received a treatment of AL/RSV/Fe_3O_4-NPs under an applied magnetic field and the RSV content in the tumor was 6.7 and 6.2 times higher than the treatment with free RSV after 24 h and 48 h, respectively. Importantly, in the *in vivo* biodistribution studies, the AUC of AL/RSV/Fe_3O_4-NPs increased 6.0 and 2.2 times relatively to free RSV and RSV-loaded lignin NPs without Fe_3O_4 (AL/RSV-NPs), respectively, confirming the EPR effect of the NPs beyond the applied magnetic field. The *in vivo* anticancer efficiency of AL/RSV/Fe_3O_4-NPs was conducted in LLC tumor-bearing mice and it was compared with PBS and empty AL-NPs, which exert no significant effect. Contrary, the AL/RSV/Fe_3O_4-NPs significantly inhibited the tumor growth with no systemic toxicities (Fig. 5). This improved anticancer activity could be due to the sustained release of RSV and its accumulation in the tumor granted by the AL/RSV/Fe_3O_4-NP small particle size, as well as by the applied magnetic field capable of guiding RSV to the target tumor site. Moreover, the AL/Fe_3O_4-NPs were able to overcome the poor solubility and light sensitivity of RSV inducing a good biocompatibility and no hypersensitive reactions. Foremost, AL/RSV/Fe_3O_4-NPs, composed mainly by natural products, proved to be a safe, efficient and promising formulation to be used as an anticancer therapeutic strategy (105).

OUTSTANDING CONCERNS

The formerly presented nanotherapeutic strategies proved to be able to enhance RSV solubility, biodistribution and anticancer bioactivity. Nevertheless, the use of NPs as *in vivo* delivery carriers for RSV might pose some challenges, essentially due to the poor stability of certain types of NPs, e.g., polymeric micelles (122). Furthermore, several limitations regarding the drug loading capacity of NPs are identified as well. For instance, SLNs, despite being a biocompatible and a cost-effective delivery approach, show a poor drug loading capacity (102). In contrast, polymeric NPs present reasonable drug loading values; however, their production and scaling-up processes are meticulous, demanding and expensive (37). In addition, it is a rough quest to find biocompatible and safe polymers. Meanwhile, for that reason, the use of natural and biocompatible materials to produce NPs, such as albumin (120) and lignin (105), has been increasingly exploited by the scientific community.

The delivery efficiency and the long-term safety of NPs are still challenging (37). Particularly in cancer, improving the delivery efficiency is crucial, since when administered NPs face several physical (e.g., diffusion and aggregation) and biological barriers (e.g., mononuclear phagocytic system (MPS) and renal clearance pathways). These barriers affect the percentage of NPs reaching the target tumor tissue and, subsequently, reduce the biodistribution, bioavailability and the anticancer therapeutic effects of the encapsulated or

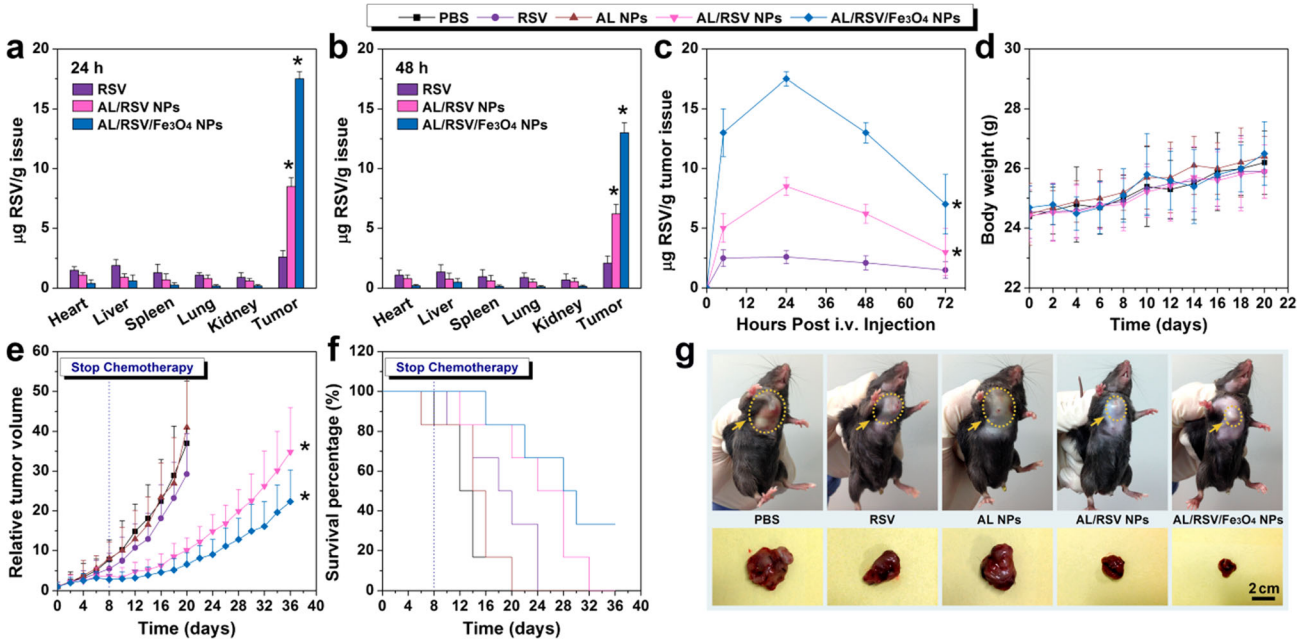

Fig. 5. Tissue distribution with the different RSV formulations for 24 h (**a**) and 48 h (**b**). RSV content in tumor issues post i.v. injection (**c**). Body weight (**d**), tumor inhibition (**e**), and survival rates (**f**) of tumor-bearing mice with different formulations. Tumor photographs from different groups on day 20 (**g**). Reprinted with permission from (105). Copyright (2019) American Chemical Society. AL NPs alkali lignin nanoparticles; AL/RSV NP resveratrol-loaded alkali lignin nanoparticle; AL/RSV/Fe3O4 NP iron oxide magnetic resveratrol-loaded alkali lignin nanoparticle; i.v. intravenous; PBS phosphate-buffered saline; RSV resveratrol

conjugated drug. Moreover, failures in the NP internalization by cancer cells may lead to accumulation and, consequently, toxicity problems on normal cells. Thereby, it is mandatory to pursue strategies that may enhance the NP delivery efficiency and safety. As described in this review, steps are already being taken in that direction, as demonstrated in Guo *et al.* (107) study that reports the use of a ligand, Tf, to enhance the glioma cellular uptake of PEG-PLA-NPs conjugated with RSV; or in Dai *et al.* (105) work that refers the use of magnetic RSV-loaded lignin NPs (AL/RSV/Fe_3O_4-NPs) to target lung cancer. In this context, it becomes imperative the application of multi-parametric studies concerning the NP transportion into and through tumors. Towards that aim, it is foresee that new tools based on computational and mathematical models will provide a better understanding of the delivery process and how it can be improved in both preclinical and clinical stages.

It is clear the necessity to test RSV-NPs in *in vivo* models in order to enable the clinical translational progress. In the future, cross-animal analysis using optimal NPs to target cancer is essential to identify the underlying delivery mechanisms, thus enabling the development of functionalized and target-specific NPs that will certainly enhance the delivery efficacy. Additionally, it is indispensable to test if there is an improvement in the known RSV chemotherapeutic and chemopreventive properties and/or a reduction of RSV toxicity on normal cells and, only afterwards, consider RSV-NPs as a viable technological alternative to deliver RSV.

RSV-NPs may constitute potential alternative chemotherapeutic strategies; however, it is important to underline that to reach that possibility, the NP production and scaling up need to fulfill the process and legal requirements of the pharmaceutical industry. Moreover, the efficacy and safety of the RSV-NPs has to be confirmed in preclinical and clinical trials, having in mind the dosage, route of administration and tumor origin (6,109). Thus, it is predicted that the line of research that contemplates the use of RSV-NPs in cancer will surely meet new developments in the near future.

The consulted literature indicates that even though nanotechnology is an attractive strategy to improve RSV cancer therapeutics, further efforts should be putforth in order to allow RSV-NPs clinical translation.

CONCLUSIONS

RSV is an actively researched phytochemical due to its recognized wide spectrum of bioactivities, exhibiting a great potential as an anticancer agent due to its pleotropic action. Moreover, RSV metabolism generates several derivatives that, similarly to RSV, are being identified to have potential bioactivities, which constitutes a key topic for further studies.

As reviewed, NPs are a feasible technological strategy to surpass RSV physicochemical and metabolization hurdles, thus improving its biodistribution and pharmacokinetics in normal and cancer tissues. So far, a myriad of RSV-NPs with different physicochemical properties (i.e., particle size, shape) and surface modifications have been produced and administered in *in vitro* and in *in vivo* cancer models. However, the transition of cellular to *in vivo* tests is cumbersome due to factors such as the RSV concentrations and NPs safety. Moreover, the administration of different RSV-NPs to *in vivo* models focus essentially on the effects on the tumor growth, lacking information regarding the RSV pharmacokinetics and pharmacodynamics. As described, RSV interacts

with several molecular targets; thus, further investigations are deemed necessary to comprehend the molecular mechanisms behind the anticancer *in vivo* therapeutic action of RSV and RSV-NPs. The extensive work carried out in cancer nanomedicine unveil a tendency to raise the NP complexity, i.e., to modify the NPs attributing new biological or medical functions. For instance, the authors have emphasized the development of NPs capable of producing heat or ROS upon light irradiation to eradicate cancer cells (consisting of photodynamic and photothermal therapies); the creation of NPs able of releasing drugs upon a pH or enzymatic reaction; or the adaptation of contrast agents able of permitting the visualization of NP intake combining diagnostic and therapy (theranostics). Furthermore, NPs enable the use of combined therapy, i.e., the co-loading of different drugs with synergetic therapeutic effects in a single structure. Towards this aim, RSV may be combined with other phytochemicals, e.g., curcumin, or inclusive with additional conventional chemotherapeutic agents, such as Tem and doxorubicin hydrochloride (DH), despite the need to test these synergisms in distinct cellular and animal models. Nevertheless, regardless all the benefits linked with NPs, the shortage of studies regarding their long-term anticancer efficacy, toxicity and safety effectively delay the progression to preclinical and clinical trials, as well as the NPs-based product commercialization.

It is the authors insight that this line of research, which exploits NPs as carriers to deliver phytochemicals with anticancer therapeutic interest, namely those referring to RSV, will suffer breakthroughs essentially due to the complex cancer cell's biology and multidrug-resistant phenotype. In fact, in cancer therapeutics, there is a constant pursue for innovative therapeutic technologies and currently the focus is on nanomedicine. Even so, the studies cited in this review constitute the first strides that support the potential of RSV-NPs as a solid nanomedicine approach for cancer therapy. It is foreseen that an increase on the quantity and quality of *in vivo* investigations regarding RSV-NPs against cancer will incite the outbreak of further scientific evidences that will enable RSV-NPs clinical translation and possibly, in the future, their trading.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors declare that they have no competing interests.

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