Commentary

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# Resveratrol Promotes Self-digestion to Put Cancer to Sleep

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Resveratrol, a natural polyphenol present in a variety of food stuff, has been shown to exert preventive and curative anticancer activity in several in vitro and in vivo models. Such chemopreventive/anticancer activity has been linked to biochemical and epigenetic modifications of multiple pathways involved in carcinogenesis and metastasization. In this commentary, we focus on the recent work done in our laboratory showing that resveratrol has potential to prevent and cure cancer by promoting epigenetic-mediated autophagy-dependent tumor dormancy, an effect associated with re-education of the cancer-associated fibroblasts and reduced production of inflammatory cytokines in the tumor microenvironment. The clinical translation of the current knowledge on resveratrol anticancer activity is also discussed.

**Key Words** Natural products, Dormancy, Autophagy, Cytokines, Tumor microenvironment

## INTRODUCTION

*Trans*-resveratrol (3,4,5-trihydroxystilbene; RV), a natural polyphenol particularly enriched in berries and grapes, has been proven an effective nutraceutical for cancer prevention and treatment in several types of malignancy, both in in vitro and in vivo preclinical models [1,2]. The efficacy of RV in inhibiting cancer growth can be attributed to its ability to target multiple pathways [1,3,4]. To mention a few, RV has been shown to induce autophagy, apoptosis and autophagy-associated apoptosis in ovarian and colorectal cancer cells [5-8]. These led to inhibition of epithelial-mesenchymal transition and migration of ovarian cancer cells [9], inhibition of reduced glucose uptake and metabolism in ovarian cancer cells [10], or suppression of the insulin like growth factor 1 receptor/Akt/Wnt-β-catenin pathways in colon cancers [11] and in stem-like breast cancer cells [12]. RV regulates many of these pathways through epigenetic mechanisms [13]. For instance, we have shown that RV can modulate microRNAs to rescue the re-expression of aplasia Ras-homolog member I (ARH-I; aka DIRAS3), an imprinted tumor suppressor that positively regulates BECLIN-1-dependent autophagy [9].

Interestingly, two independent studies have shown that RV can also interrupt the malignant cross-talk between cancer-associated fibroblasts (CAFs) and cancer cells in the tumor microenvironment [14,15]. RV could inhibit breast cancer cell proliferation and migration and the stem-like properties that were promoted by CAFs, and this effect was due to inhibition of the expression of cyclin D1 and c-Myc, and of the STAT3 (a transcription factor triggered by interleukin [IL]-6), Akt and self-renewal pathways [14]. In our study, the conditioned medium of CAFs pre-treated with RV lost the ability to induce proliferation and invasiveness of cholangiocarcinoma cells [15]. The anticancer activity of RV has been proven in several preclinical in vivo models, including breast cancer [16,17], colorectal cancer [18], and lung cancer [19]. RV alone [19] or in combination with curcumin and quercetin [17] showed the ability to also modify the tumor microenvironment, reversing the infiltration of immunosuppressive Th2 lymphocytes, tumor-associated N2 neutrophils and tumor-associated M2 macrophages.

## MAIN SUBJECTS

We have previously shown that RV acts as a protein-restriction mimetic to induce mTOR-dependent autophagy, a self-digestion lysosomal-mediated process which oversees cellular homeostasis [8]. Recently, we found that reduced overall survival of cholangiocarcinoma patients was associated with low expression of autophagy markers in cancer

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cells along with high expression of IL-6 and high infiltration of CAFs in the tumor microenvironment [20]. By pre-treating cholangiocarcinoma-derived CAFs with RV in vitro, we could inhibit the secretion of IL-6 so that their conditioned medium lost the ability to repress autophagy and to induce proliferation and migration of the exposed cholangiocarcinoma cells [15]. This prompted us to test in vivo the preventive and curative potential of RV. To this end, we set three groups of nude mice in which we transplanted subcutaneously human cholangiocarcinoma cells. Mice in the first group (that had not received any treatment for five weeks) served as controls, the mice in the second group were treated with 50 mg/kg RV in drinking water two weeks before tumor transplantation and for the next five weeks, and they served as the "preventive" group, and the mice in the third group started the RV treatment one week after tumor transplantation and served as the "curative" group. The results can be summarized as follows: (i) in controls, the tumors reached an average volume of 5.00 $mm^3$ after 5 weeks in all the five mice, while in the "preventive" group, only three mice developed a tumor with an average volume of 0.15 $mm^3$, and in the "curative" group the five mice developed a tumor with an average volume of 1.0 $mm^3$; (ii) both in the "preventive" and "curative" groups, the infiltrate of CAFs and the presence of stromal IL-6 were drastically decreased; (iii) in the tumors from both the "preventive" and the "curative" groups, the autophagy markers microtubule-associated protein 1 light chain 3 (LC3) and BECLIN-1 were highly expressed [21].

It has been reported that RV can act epigenetically to modulate autophagy in cancer [22-24], and it is known that the secretions in the tumor microenvironment can epigenetically modulate autophagy to drive tumor dormancy [25]. In a separate study, we demonstrated that RV could keep ovarian cancer cells grown as 3D spheroids (a proxy of peritoneal metastatic colonies) in a dormant state even in the presence of IL-6, and this effect was due to the down-regulation of miR-1305 and concomitant re-expression of its target ARH-I (DIRAS3), which then rescued BECLIN-1-dependent autophagy [26]. By extracting the data from TCGA, we found that cholangiocarcinomas highly expressing *BECN1* also showed high levels of *DIRAS3* and *CDKN1A* mRNA, strongly suggesting a dormant state of the tumors [21]. This prompted us to check for the expression of ARH-I (DIRAS3) and autophagy and dormancy markers in the biopsies of the preclinical model of transplanted cholangiocarcinomas in nude mice described above. To this end, we searched for co-expression of the autophagy markers LC3 and BECLIN-1 with p21, the marker for cell cycle arrest encoded by *CDKN1A*, and with ARH-1/DIRAS3 (encoded by *DIRAS3*), a protein playing a pivotal role in the switch from autophagic cell death to autophagy-driven cell dormancy [27]. The images in Figure 1 show that RV-treated tumors, either via the preventive or the curative protocol, present high levels of LC3 and p21 co-expression, suggestive of the involvement of autophagy in cell cycle arrest. Further, RV greatly enhanced the expression of DIRAS3, which was shown to largely interact with BECLIN-1. To be noted, in RV-treated tumors, the expression of STAT3 (which is downstream of IL-6 signalling) was decreased, and this protein was found largely co-localized in the cytoplasm bound to DIRAS3. Taken together, these data suggest induction of a dormant state in association with induction of autophagy in the tumors exposed to RV.

Cancer develops following genetic and epigenetic alterations of the genes controlling cell cycle and cell proliferation, telomerase activity and stemness, cell metabolism, motility and invasiveness, autophagy, apoptosis, and other types (e.g., ferroptosis) of cell death, along with the complicity of the tumor microenvironment which promotes angiogenesis and inhibits the immune response [28]. Chemotherapy is the gold standard for cancer treatment, but unfortunately it has several drawbacks that discourages the patients from continuing the therapy [29]. Disappointingly, even precision therapy with molecular drugs targeting "driver" oncogenic proteins has a limited efficacy [30,31]. RV could represent a valid alternative

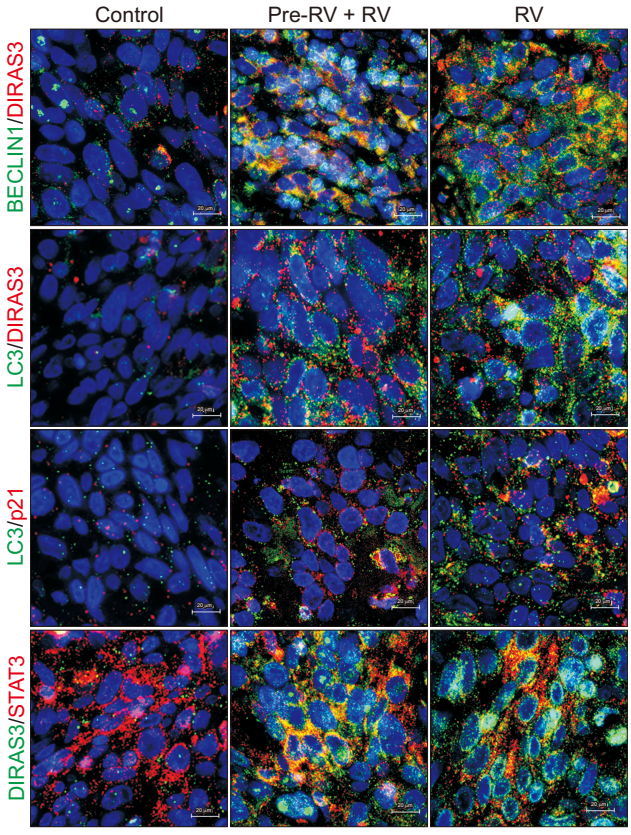


**Figure 1. The immunofluorescence co-staining for LC3/p21, BECLIN-1/DIRAS3, LC3/DIRAS3 and DIRAS3/STAT3 shows up-regulation of autophagy and autophagy-dependent dormancy in Pre-RV + RV and RV-treated mice (for treatment details see the text and reference [21]).** RV, resveratrol; LC3, light chain 3. Scale bar = 20 μm.

and/or adjuvant therapy for synergizing with chemotherapeutics and at the same time mitigating their undesired side effects [32]. Two characteristics make RV an attractive cancer therapeutic worth of further investigations: (i) it is a hormetic drug, meaning that it elicits different effects in normal and diseased tissues depending on the concentration [33], and (ii) it causes negligible, if any, side effects [34]. Moreover, a recent systematic drug-target interaction network study found that RV can target up to 23 driver genes (including *mTOR*, *BRCA1*, *TP53*, *PTEN*, *HGF*, *CTNB1*, *CDKN1A*, and *AKT1*, among others) significantly mutated in up to 15 types of cancer [35]. Yet, clinical trials with RV (available at https://clinicaltrials.gov/ct2/) appear not to meet the expectations, likely because of the low bioavailability and rapid excretion of RV, which prevent it from reaching a pharmacologic concentration in the target tissue [36].

It is indeed puzzling that despite the vastness of in vitro and in vivo preclinical data supporting its anticancer activity, the clinical efficacy of RV in cancer patients remains ambiguous and inconclusive [37]. Possible reasons for the failure of the clinical trials include mistakes in the experimental design (time, dose, administration route, patient's selection criteria, etc.), the supposed mechanisms of action, and the endpoints, which are based on in vitro and in animal studies [36,38]. First, the assumption that high dose administration is needed to obtain the desired therapeutic effect in the diseased organ has been challenged by a recent study showing that low dietary administration of RV (5 mg) is more effective than a pharmacologic dose (1 g) for a chemopreventive activity in colorectal cancer [38]. Interestingly, the chemopreventive effect was influenced by the diet, and it was associated with increased expression of LC3 and p21 (indicative of autophagy-mediated arrest of cell proliferation) in human colonic mucosa [38]. To be noted, the oral administration of 5 or 50 mgRV twice daily for 12 weeks in breast cancer women led to hypomethylation and re-expression of the *RASSF-1α* tumor suppressor gene encoding a RAS-associated protein that inhibits cell cycle and promotes apoptosis [39].

Regarding the mechanisms of action, it is conceivable that the pharmacologic inhibition of biochemical pathways attained in vitro at a relatively high concentration (10 to 200 μM) of RV unlikely occurs in patients, where its plasma concentration reached 20 nM after oral administration of 360 μg/kg [40]. As a comparison with the mouse, 15 mg/kg of RV administered in drinking water (0.01%) would lead to a putative peak in the plasma of 1.5 μM [36]. Another possible reason for the different outcome at a given dose in animal experiments and clinical trials is that orally administered RV synergizes with microbiota metabolites to exert anticancer activity via yet unidentified pathways [41]. The microbiota in rodents and in humans are different and influenced by the diet, and RV itself can modify the microbiota [41]. This fact may account for the difference in the dose-response between the two species. This hypothesis is being explored in our laboratory.

The dose of RV (50 mg/kg) used in our nude mice model (which would lead to a putative peak in the plasma of 5 μM) might be equivalent to approximately 500 to 600 mg per 60 to 70 kg individual. Based on our findings, we hypothesize that for curative purposes, oral administration of RV at 10 to 20 mg/kg (approximately 600 to 1,400 mg per 60 to 70 kg body weight) daily eventually leads to epigenetic changes of the gene expression and metabolism both in cancer and stromal

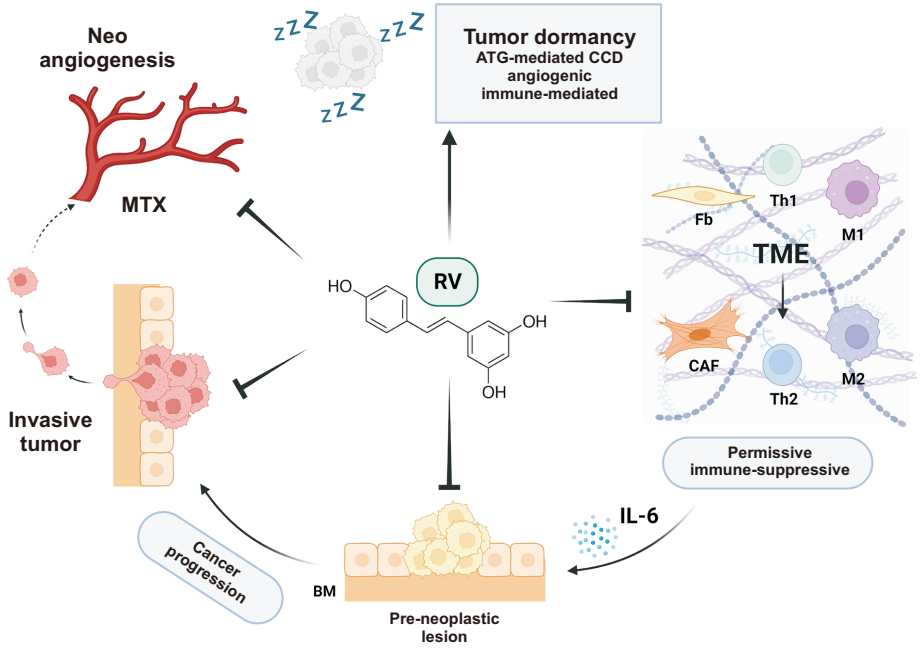


**Figure 2. The cartoon illustrates the action of RV in the tumor microenvironment comprising fibroblasts and immune cells as well as the cancer cells which eventually leads to tumor dormancy.** ATG, autophagy; CCD, cancer cell dormancy; MTX, metastasis; RV, resveratrol; TME, tumor microenvironment; Fb, fibroblasts; Th, T CD4 lymphocyte; M, macrophages; CAF, cancer-associated fibroblasts; BM, basement membrane; IL, interleukin.

cells. This can restore homeostasis and tumor dormancy in the affected tissue. Of course, the dose must be personalized to the patient, taking into account sensitive factors such as age, tumor stage, performance, diet, lifestyle, and concomitant therapies with drugs and/or other natural products.

As per the treatment endpoints, it is to be stressed that in our animal models treated with RV in drinking water, we obtained an effect not only in cancer cells (where markers of autophagy, apoptosis, cell cycle arrest and cell dormancy were induced) but also in the tumor microenvironment (where infiltration of CAFs and stromal IL-6 levels were decreased) [21]. In a separate study, we showed that RV also reduces angiogenesis and infiltration of immune-suppressive cells in the tumor microenvironment of transplanted lung cancer cells [19]. The latter finding is compatible with induction of angiogenic and immune-mediated tumor dormancy. The multiple processes targeted by RV leading to tumor dormancy are schematically summarized in Figure 2.

## CONCLUSION

In summary, the body of scientific evidence reported here demonstrates that RV can block tumor growth by inducing “drowsiness” of tumor cells and modifying the microenvironment to create a “comfortable bed” to put the tumor to sleep.

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## CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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