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Review

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Control of MicroRNA Expression as a New Way for Resveratrol To Deliver Its Beneficial Effects

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ABSTRACT: Grapes produce large amounts of polyphenols. Many of them accumulate in the skin, pulp, and seeds and are consequently found in wine. The health benefits of a moderate consumption of wine have been attributed at least in part to grape's polyphenols. Among them, resveratrol (3,5,4′-trihydroxystilbene) is a phytoalexin that stimulates plant cell defenses against infections and also plays protective roles in humans, where it delays cardiovascular alterations and exerts anticancer and anti-inflammatory effects. Despite numerous studies, the molecular mechanisms of resveratrol action are only partially understood. Given its pleiotropic effects, it was previously suggested that resveratrol protective properties may arise from its modulation of the expression of microRNAs. Therefore, this review will focus on the effects of resveratrol on microRNA populations in humans and human cell lines, especially emphasizing the microRNAs that have been implicated in resveratrol effects on inflammation, cancer, metabolism, and muscle differentiation

KEYWORDS: *microRNAs, resveratrol, cancer, inflammation, metabolic disease, muscle, grape, wine*

INTRODUCTION

Grape plants (*Vitis vinifera*) produce substantial amounts of a wide variety of polyphenols as secondary metabolites. Many of them accumulating in the skin, pulp, and seeds of grapes are subsequently found in grape juice or wine. The health benefits of a moderate consumption of wine have now been firmly established[1] and are due at least in part to their high content in polyphenols, especially resveratrol (3,5,4′-trihydroxystilbene), the concentration of which is particularly elevated (3−5.5 mg/L) in wines prepared from Gamay and Pinot noir plants.[2] Resveratrol is a phytoalexin that stimulates plant defense against various biotic and abiotic stresses and also plays protective roles in animals. It is also found in other comestible berries and seeds (blueberries, peanuts, ...) as well as in several medicinal plants (*Polygonum cuspidatum*, *Yucca schidigera*, ...). In humans, resveratrol is believed to delay or limit cardiovascular alterations, cancer, inflammation, and related pathologies such as chronic inflammation.[3] Despite a number of studies that investigated several signaling and transcriptional pathways, the mechanisms of the pleiotropic action of resveratrol remain poorly understood presently.[4] However, several recent publications have established that one reason resveratrol can affect so many different regulatory pathways is due to its capability to modulate the expression, and consequently the regulatory effects, of a number of small noncoding RNAs, namely, microRNAs (miRNAs).[5]

Excellent reviews[6,7] have recently described resveratrol effects in animal models. In contrast to "classical" coding transcripts, noncoding RNAs have been generally much less conserved during evolution. This review will focus on describing resveratrol effects on miRNA populations in humans and human cell lines. After exposing the principal results drawn from these studies, we will propose how, in our view, resveratrol might be beneficial not only at high doses (i.e., at pharmaceutical doses or as a food supplement) but also at much lower doses such as those provided by a regular diet intake.

GENERAL PROPERTIES OF RESVERATROL

Polyphenols are important plant microcomponents in terms of both quantity and diversity. In particular, grape plants produce substantial amounts of a wide variety of polyphenols as secondary metabolites, with many of them accumulating in the skin, pulp, and seeds of grapes and being found in grape juice or wine following fermentation. The health benefits of a moderate consumption of wine have been attributed to grape's polyphenols: procyanidins, flavonoids, phenolic acids, and stilbenoids.[8] However, the molecular bases of these benefits have not yet been fully elucidated. Resveratrol is a phytoalexin that stimulates cell defenses in plants.[9] The *trans* (*E*) isomer of resveratrol is the most abundant and active form of resveratrol as compared to the *cis* (*Z*) isomer. In the grape, resveratrol mainly accumulates in a glycosylated conjugated state (piceid).[10] Some dimethoxylated derivatives are also present (pterostilbene) as well as resveratrol oligomers (ε-viniferin, a dimer, and hopeaphenol, a tetramer).[11] Resveratrol has been established as a powerful antioxidant with a direct impact on oxidative stress. Resveratrol has also been shown to exert a

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variety of anti-inflammatory effects through the inhibition of lipoxygenases and cyclo-oxygenases that synthesize pro-inflammatory mediators from arachidonic acid, protein kinases such as PKCs and PKD, receptor tyrosine kinases, and lipid kinases, as well as IKKα, an activator of the pro-inflammatory NF-κB pathway.[5] In addition, resveratrol regulates apoptosis[12] and cell cycle progression[13] and down-regulates the MAP kinase signaling pathway, the NF-κB pathway, and the activator protein-1 (AP-1) pathway. Due to its pleiotropic antiproliferative activities, resveratrol has been in recent past years at the stage of preclinical studies for cancer prevention[6,7] and is presently at the onset on clinical studies.[14−18]

The surprisingly great number of beneficial effects of resveratrol have led to the hypothesis that this compound should modulate the expression of some global regulators. Indeed, it has been recently established that resveratrol can modulate the expression of miRNAs[5] (see below).

DIETARY RESVERATROL VERSUS RESVERATROL-ENRICHED NUTRIENTS: BIOAVAILABILITY AND BIOACTIVITY

With regard to resveratrol bioavailability, it is largely admitted that resveratrol is rapidly metabolized into a conjugated forms (glucurono- or sulfo-) or into a hydroxylated component (piceatannol) and is found only at low plasmatic levels. Its metabolites could possibly exhibit bioactivity (piceatannol) or be recycled (sulfoconjugated resveratrol). Interestingly, a recent study on resveratrol intake by volunteers showed resveratrol plasmatic levels in the micromolar range.[15] This concentration was compatible with those required for resveratrol binding to and inhibition of enzymes such as cyclo-oxygenase 1 (COX1) and COX2[19] or the integrin $\alpha V\beta 3$ receptor.[20] It has also been suggested that resveratrol concentration in rat blood may not always reflect resveratrol levels in peripheral tissues because high resveratrol concentrations have, for example, been found in the liver.[21] In addition, we have shown that resveratrol can accumulate in hepatic cells not only through diffusion but also through active carrier-mediated uptake.[22] Accordingly, in colorectal cancer patients, resveratrol and resveratrol metabolites were found to accumulate in the colon at concentrations often exceeding those that have been widely reported as having activity in numerous preclinical systems.[16,18] Of note, resveratrol and resveratrol metabolites were higher in the right side of the colon compared to the left.[16] However, whereas high levels of resveratrol are achievable in the colon, resveratrol levels in other tissues are expected to match more closely the concentrations in plasma; that is, resveratrol effects in the other organs may be more dependent on the activity of resveratrol metabolites. Recent data showed that resveratrol monosulfate and bisulfate derivatives display biological activities compatible with antitumor affects, such as the inhibition of COX1, COX2, NO production, and *iNOS* expression, or the activation of SIRT1.[19,23] Accordingly, new synthetic derivatives with improved anti-inflammatory and antitumor properties have been recently tested.[24]

On the other hand, the recent discoveries (see hereafter) that some of resveratrol's beneficial effects are due to its regulation of miRNA expression[5] and that miRNAs can travel from one cell to another through the blood[25] open the exciting possibility that the protective effects of resveratrol may be delivered to a large range of tissues through bloodborne miRNAs.

So far, epidemiological studies on the effects of dietary resveratrol in human populations have been rare because the regular intake of resveratrol is often associated with the consumption of alcoholic drinks, which can result in harmful effects. Nevertheless, earlier studies showed that the regular consumption of wine polyphenols including resveratrol leads to a decreased mortality and morbidity by vascular diseases compared to the consumption of beer or spirits.[26] More recently, a case control study on the prevention of breast cancer showed epidemiological evidence that resveratrol from grape is inversely related to breast cancer risk.[27] These promising results warrant further studies, especially to determine the concentration of resveratrol that could exert protective effects when used as nutrient supplementation.

MICRO-RNAS AS NEW PLAYERS IN THE REGULATION OF CELL HOMEOSTASIS

MiRNA function in the cell is a tremendously expanding new research area. The first noncoding small regulatory RNA (*lin4*) was identified in 1993 as a developmental regulator in *Caenorhabditis elegans* by Dr. V. Ambros's team.[28] The seminal discovery by Dr. C. M. Croce's group that some miRNAs are deleted in cancer[29] and are therefore potential players in cancer induction or progression subsequently drew the attention of many scientists, and the area of miRNAs and cancer has blossomed ever since. MiRNAs were rapidly shown to be present not only in animals but also in plants and viruses. Since then, miRNAs have been implicated in the regulation of cell proliferation, differentiation, and homeostasis, as well as in the innate and adaptative immune responses. To date, some 1500 miRNAs have been identified in humans. MiRNAs are produced from long capped and polyadenylated primary transcripts (pri-miRNAs). In the nucleus pri-miRNAs are cleaved by the RNase III Drosha, and the resulting pre-miRNAs are subsequently exported to the cytoplasm by Exportin 5. Further maturation of pre-miRNAs to miRNAs occurs in the cytoplasm through the RNase III Dicer. Mature miRNAs are loaded onto a ribonucleoprotein complex known as the RISC (RNA-induced silencing complex) through their interaction with the Ago2 (Argonaute) protein, subsequently allowing them to target mRNAs by complementary base pairing. MiRNAs usually target the 3′-untranslated region (3′-UTR) of transcripts that contains their consensus sequence (i.e., a short sequence complementary to their so-called seed region). Their binding to mRNAs within the RISC triggers the target transcripts for either degradation or translation inhibition, consequently decreasing the levels of the encoded proteins. The consequences are multiple. For instance, miRNAs can modulate cell signaling by changing the level of kinases or phosphatases, regulate transcription by unbalancing the ratios of transcription factors, or modify epigenetic marks by targeting methyltransferases/demethylases and histone acetylases/deacetylases. As a consequence, the mutation of genes encoding some miRNAs or that of target sequences to tumor-suppressor miRNAs in the 3′-UTR of transcripts can initiate oncogenic processes.[29]

Interestingly, miRNA misexpression has been linked with major pathologies such as cancer or cardiovascular, neurodegenerative, and autoimmune diseases.[30] Finally, miRNAs have recently been found in blood and other body fluids. They have been shown to be transported from cell to cell either through gap junction or through blood secretion and to exert their targeting capabilities in recipient cells. In blood, miRNAs have been found either in microvesicles, exosomes, HDLs (high density lipoproteins) or associated with RNA-binding proteins

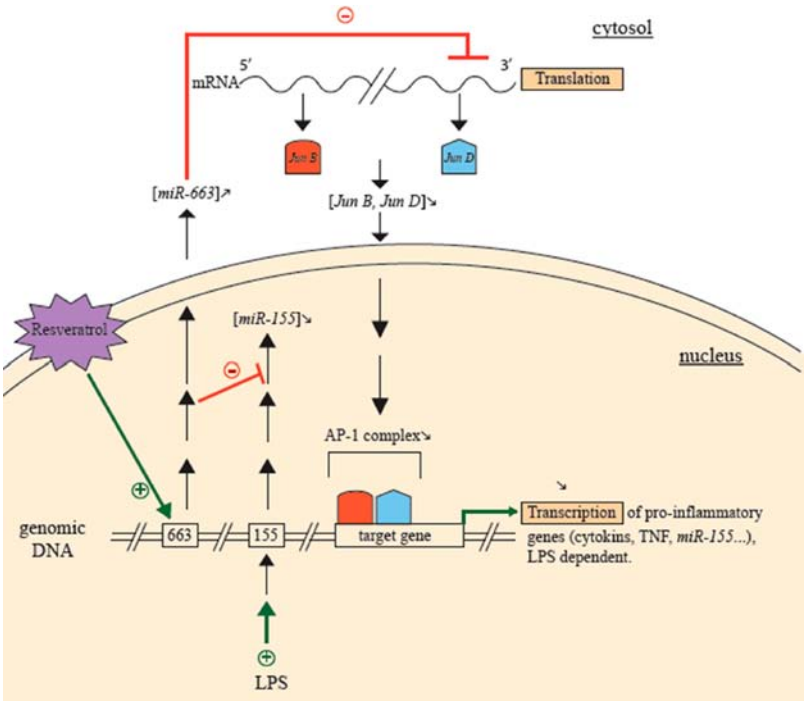

Figure 1. Resveratrol exhibits anti-inflammatory properties, at least in part by modulating the expression of *miR-663* and *miR-155* miRNAs (adapted from the results of Tili et al.[32]). In THP-1 human monocytes, resveratrol down-regulates pro-inflammatory *miR-155* level through the up-regulation of *miR-663*, an anti-inflammatory miRNA targeting *JunB* and *JunD* transcripts. JunB and JunD are components of the pro-inflammatory and oncogenic transcription complex AP-1. LPS, bacterial lipopolysaccharides; 155, *Bic/MHG2* gene containing *miR-155* precursor; 663, *LO284801* locus containing *miR- 663* precursor; JunB, JunD, proteins of the AP-1 complex (activating protein-1) stimulating the transcription of pro-inflammatory genes; target gene, pro-inflammatory and other genes.

such as Ago2 or nucleophosmin 1.[25] MiRNAs are now believed to act as proto-hormones capable of delivering an effect in distant cells, which could be responsible for the induction of metastases at a distant location from the original tumor.[25] In contrast, it is probable that some pharmaceutical compounds, including resveratrol, may possibly exert wide anti-inflammatory and antitumor effects in the body by inducing the secretion of anti-inflammatory and antitumor miRNAs in the blood.

MiRNAs can potentially target tens to hundreds of different transcripts. Given that many of those encode transcription factors or factors involved in different signal transduction pathways, miRNAs can also indirectly regulate the expression of a number of nontarget transcripts. Therefore, the comparison of miRNA signatures in normal and pathologic tissues is of tremendous interest because these molecules offer a higher potential than coding transcripts for diagnosis or prognosis purposes. Furthermore, the development of new treatments based on the targeted expression or inhibition of miRNAs of interest is conceivable. In this respect compounds capable of increasing the expression of "good", anti-inflammatory and tumor-suppressor, miRNAs and at the same time decreasing the expression of "bad", pro-inflammatory and oncogenic miRNAs, provide hope for the development of new treatments against a number of pathologies. Moreover, when molecules such as resveratrol are present in nutrients and can be absorbed at low dose on a regular daily basis, they offer the further prospect to be used as preventive agents.

MODULATION OF MICRO-RNA POPULATIONS BY RESVERATROL: BIOLOGICAL CONSEQUENCES

Resveratrol Regulates the Expression of MiRNAs Implicated in Inflammation. Inflammation is a complex immune response to pathogens, allergens, damaged cells, tissue injury, or toxic molecules. This normally beneficial and self-contained inflammation may become chronic. Chronic inflammation has been linked to many pathologies such as vascular alterations, neurodegenerative diseases, rheumatoid arthritis, chronic asthma, multiple sclerosis, inflammatory bowel disease, and cancers. It has been established[31] that inflammation is associated with the induction or the aggravation of ≥25% of cancers, especially colorectal cancers. Interestingly, some polyphenols, including resveratrol, are known to exhibit anti-inflammatory properties, and we recently showed that resveratrol can regulate the expression of both pro- and anti-inflammatory miRNAs.[32] Thus, in human THP-1 monocytic cells as well as in human blood monocytes, resveratrol up-regulates *miR-663*, an anti-inflammatory and tumor-suppressor miRNA that decreases AP-1 transcriptional activity and impairs its up-regulation by lipopolysaccharides (LPS) at least in part by targeting *JunB* and *JunD* transcripts. In contrast, resveratrol impairs the up-regulation of pro-inflammatory and oncogenic *miR-155* by LPS in a *miR-663*-dependent manner (Figure 1). These results open the perspective of manipulating *miR-663* levels to potentiate resveratrol anti-inflammatory and antitumor effects at least in malignancies associated with elevated levels of *miR-155*.

Resveratrol Impairs the Expression of Oncogenic miRNAs while Up-regulating Tumor-Suppressor miRNAs. The first study of resveratrol effects on endogenous miRNA populations was conducted in a human colon cancer cell line (SW480).[33] Resveratrol proved to decrease the levels of oncogenic miRNAs (*miR-17, 21, 23a, 23b, 25, 29, 92a-2, 103-1, 103-2, 146a*, ...) that collectively target transcripts encoding Dicer1, the cytoplasmic RNase III producing mature

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miRNAs from their immediate precursors, and tumor-suppressor factors such as PDCD4 or PTEN, as well as key effectors of the TGFβ signaling pathway. In contrast, resveratrol increased the level of tumor-suppressor *miR-663*, one of the rare miRNAs that target *TGFβ1* transcripts. On the basis of these paradoxical effects it has been suggested that resveratrol, known for both its anticancer and antimetastatic properties, might possibly increase the cytostatic activity of the canonical TGFβ1 pathway at the early stages of tumors but nevertheless decrease the pro-metastasis effects of TGFβ1 (and especially its induction of epithelium-to-mesenchyme transitions) at more advanced stages of tumors, possibly through the up-regulation of *miR-663*. This study further emphasized the potential interest of manipulating the levels of key miRNAs to increase the anticancer and antimetastatic effects of resveratrol. The anticancer effects of resveratrol mediated by miRNAs in colorectal tumor-derived cells are summarized in Figure 2.

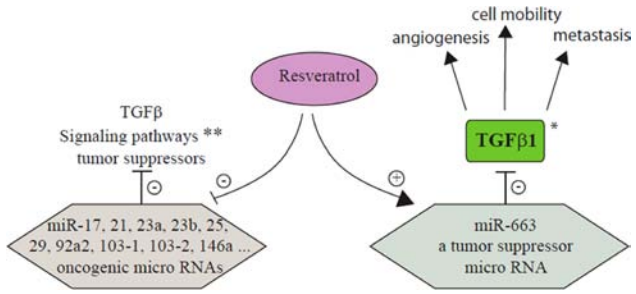

Figure 2. Resveratrol modulates the level of miRNAs targeting transcripts encoding tumor suppressors and effectors of the TGFβ signaling pathway in SW480 human colorectal cancer cells (adapted from from the results of Tili et al.[33]). *, Cytostatic at the early stages of tumor, prometastatic at later stages; **, SMAD, similar to MAD in *Drosophila* (mother against Dpp), a transcription factor that transduces the canonical TGFβ signaling following its phosphorylation by the TGFβ receptor type I. PTEN, a tumor-suppressor factor that is a member of the protein tyrosine phosphatase family; PDCD4, programmed cell death 4, a nucleocytosolic protein known as a tumor suppressor.

It was subsequently demonstrated that resveratrol treatment alters miRNA expression in human A549 lung cancer cells with 71 miRNAs exhibiting >2-fold changes in resveratrol-treated cells.[34] Furthermore, resveratrol treatment of antibenzo[*a*]-pyrene-7,8-diol-9,10-epoxide-transformed human 16HBE-T bronchial epithelial cells induced the expression of *miR-622*, a miRNA that behaves as a tumor suppressor by targeting transcripts encoding K-Ras.[35] As a consequence *miR-622* strongly suppresses the ability of 16HBE-T cells to both form colonies in vitro and develop tumors in nude mice. On the other hand, resveratrol treatment of PCa prostate cancer cells[36] decreased the expression of 23 miRNAs, including oncogenic miRNAs of the *miR-17-92* and *miR-93-106b* clusters that target transcripts encoding phosphatase and tensin homologue deleted on chromosome 10 (PTEN), a tumor-suppressor protein that is down-regulated in nearly all cancers. Another paper established that resveratrol promotes the expression and activity of Ago2, thereby inhibiting breast cancer stem-like cell characteristics by increasing the expression of a number of tumor-suppressor miRNAs, including *miR-16*, *miR-141*, *miR-143*, and *miR-200c*.[37] Finally, it was recently shown that resveratrol decreases the ability of CL1-5 and A549 lung cancer cells to migrate and invade in a dose-dependent manner. This effect is due to resveratrol down-regulation of *miR-520h*, consequently leading to the inhibition of Akt and then to a down-regulation of FOXC2, a crucial transcription factor that favors epithelium to mesenchyme transitions, and thus enhances the invasiveness and metastasis ability of cancer cells.[38]

Of note, whereas miRNAs such as *lin4* (aka *miR-125* in vertebrates) and *let-7* have been well conserved during evolution, other miRNAs such as *miR-155* or anti-inflammatory *miR-146a* and *miR-146b* are found only in vertebrates. Other miRNAs such as *miR-663* are primate-specific. Therefore, although a number of resveratrol effects have been demonstrated in rodents, only studies in human cell lines have the potential to show the precise effects of resveratrol on human endogenous miRNA populations. Rodents are close relatives to primates from an evolutionary point of view, but they have repeatedly been shown to respond in their own specific manner to the action of drugs such as thalidomide (a sedative that remained without apparent deleterious effects in pregnant rats but proved highly teratogenic in pregnant women) or to inflammation with anti-inflammatory *miR-146a* and *miR-146b* being highly up-regulated in humans but not in mice. Rodents might thus not represent an adequate system to predict the efficiency or toxicity of tested compounds, especially when it comes to their effects on endogenous miRNA populations. For example, *miR-146a* is specifically up-regulated in the brain of Alzheimer's patients, and this up-regulation is linked with the down-regulation of complement factor H (CFH), an important repressor of the inflammatory response in the brain.[39] Due to the lack of strong up-regulation of *miR-146a* in inflammatory conditions in mice, it appears that mouse models of Alzheimer's disease might not reflect all potential beneficiary effects of resveratrol on this type of pathology.

Resveratrol Modulates Heart and Skeletal Muscles Functions. Muscle physiology, both cardiac and skeletal, is absolutely essential to the organism, and tremendous efforts have been made in this research area for many years. The functions of these tissues are closely dependent on nutrition and the quality of food, that is, its composition in fatty acids, carbohydrates, amino acids, etc. Food also contains a wide variety of polyphenols that are able to modulate the cardiac pump, blood vessel circulation, and physical exercise, as well as the aging of muscles. Nevertheless, except for a study on mouse physiology[40] and cardiac cells,[41] the effects of resveratrol on muscle cell metabolism have not been explored in depth.

MiRNAs have been shown to regulate and provide robustness to the myogenic transcriptional network[42] and have been implicated in various cardiovascular functions and pathologies.[43] For example, during rat heart muscle tissue remodeling, over 25 miRNAs presented a differential expression. Among them, *miR-21* was shown to regulate the ERK/MAP kinase signaling pathway and was previously implicated in cardiac remodeling.[44] However, until very recently nothing was known about the role of resveratrol in the regulation of miRNAs in muscle cells. In 2010, Mukhopadhyay et al. reported a cardioprotective effect of resveratrol and proposed miRNA signatures in a rat ischemia/reperfusion (I/R) model.[45] They reported that *miR-20b*, which modulates VEGF signaling, was down-regulated 6-fold by resveratrol. This strong down-regulation of *miR-20b* was supposed to be linked with a powerful antiangiogenic action of resveratrol in the ischemic myocardium and synergic effects of resveratrol and γ-tocotrienol. The targets of miRNAs for

which expression was reported to be affected by resveratrol included transcripts encoding factors such as metal ion-binding proteins, sodium and potassium ion channels, and transcription factors that may potentially play key roles in reducing I/R injury.

More recently, we reported that in mouse C2C12 skeletal myoblasts 26 miRNAs are up-regulated 1.35−8.59-fold by resveratrol, whereas 20 other miRNAs are down-regulated 1.5−47-fold.[46] Among miRNAs having levels that changed significantly ($p < 0.05$) following resveratrol treatment of C2C12 cells, *miR-20b* (with >800 putative target transcripts) as well as *miR-133* (with >300 putative target transcripts), a muscle-specific miRNA known to target transcripts encoding the serum-controlled factor involved in myoblast differentiation,[47] were down-regulated, whereas *miR-21* (with >730 putative target transcripts) and *miR-27b* (with >730 putative target transcripts) were up-regulated. Finally, *miR-149* (with >180 putative target transcripts, some of them encoding factors playing important roles in skeletal muscle) was also down-regulated by resveratrol. A process of miRNA-dependent muscle cell differentiation and conversion under resveratrol treatment is presented in Figure 3.

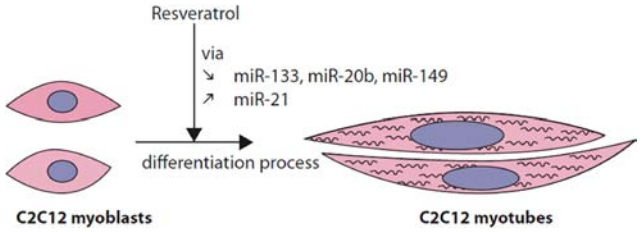

Figure 3. Resveratrol exerts pro-differentiation effects on C2C12 skeletal muscle cells via its modulation of the levels of noncoding miRNAs (adapted from the results of Kaminski et al.[46]).

Additional Cell Processes Modulated by Resveratrol in a MiRNA-Dependent Manner. Resveratrol has also been shown to behave as a potent activator of SIRT1 signaling.[48,49] This protein deacetylase is known to control energy homeostasis, fiber strength, and regeneration from damage in skeletal muscle.[50] It is therefore worth noting that *SIRT1* transcripts themselves are targeted by *miR-155* (Tili et al., unpublished results), a pro-inflammatory miRNA down-regulated by resveratrol.[32] On the other hand, *miR-30a* has been shown to target transcripts encoding Beclin 1, the mammalian homologue of the key yeast autophagy-promoting factor Atg6.[51]

POTENTIAL RESVERATROL BENEFICIAL EFFECTS

MiRNAs have recently been established as key regulators of development, cell differentiation, communication, adhesion and metabolism, cell interactions with their environment, including the response to stress or toxic compounds, and the mounting and regulation of the innate and adaptive immune responses. It is therefore not surprising that many resveratrol pleiotropic effects may implicate miRNAs. The precise mode of action of miRNAs in cells remains to be determined: especially how miRNA accessibility to its target transcripts is regulated, how different miRNAs collaborate to control the levels of the same transcripts, and in what range of miRNA and target transcript concentrations the inhibitory effects of miRNAs are effective. Nevertheless, it is already clear that miRNAs carry a higher potential than coding transcripts for diagnosis and prognosis purposes. Furthermore, different strategies have already been proposed for the utilization of miRNAs as anticancer agents in vivo. Indeed, preliminary results have been obtained not only in mice but also in humans.

As >25% of cancers have been directly or indirectly associated with inflammation and as all types of tumors are now believed to be linked with the misexpression of oncogenic and/or tumor-suppressor miRNAs, the discovery that resveratrol regulates the expression of endogenous miRNA implicated in inflammation and cancer is of primary importance: it now seems logical to associate the use of resveratrol and other natural and synthetic products with miRNA mimics or miRNA inhibitors to potentiate the anticancer and anti-inflammatory effects of these different compounds. Of note, miRNAs and other noncoding RNAs have been less conserved than coding transcripts during evolution. Therefore, given the limited toxicity of resveratrol in humans even at the highest pharmaceutical doses, only studies in humans and human cell lines may possibly offer a precise account of actual protective effects of resveratrol. Furthermore, besides their use at pharmaceutical doses, natural products such as resveratrol have the additional potential to be used as preventive agents through the intake of natural resveratrol-containing nutrients or of nutraceutical food. It will therefore be of greatest interest to develop them in conditions where bloodborne miRNA concentrations would be monitored in parallel. The recent discoveries that miRNAs can affect cells located at a distance open the exciting possibility that resveratrol may possibly deliver beneficial effects in organs where its concentration may remain low through the increase of the secretion of anti-inflammatory or tumor-suppressor miRNAs.

Of note, mice are short-lived organisms and have shown many significant differences with humans in the epigenetic regulation of gene expression, the mechanisms of X-chromosome inactivation, the nature of imprinted genes, or certain modalities of the immune response. Mice may thus not be very well suited for the analysis of the protective effects of resveratrol against tumors that can sometimes take several tens of years to develop in humans. Also, it is still not clear whether increasing sharply the expression of tumor-suppressor miRNAs during a short period of time might be better than increasing slightly their levels over an extended period, which may be expected to occur following the regular intake of low doses of natural products such as resveratrol. In any case, the many protective effects of resveratrol offer the perspective of preventing or at least partially reversing muscular dysfunctions and pathologies linked to inflammation, cancer, and autoimmune diseases when used either at pharmaceutical doses or at diet doses.

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■ REFERENCES

(1) Rifler, J.-P.; Lorcery, F.; Delmas, D.; Prost, M.; Durand, P.; Riedinger, P.; D'Athis, P.; Hudelot, B.; Ragot, K.; Limagne, E.; Mazué, F.; Lizard, G.; Latruffe, N. Influence of red wine intake in secondary prevention of myocardiac infarct patients. *Mol. Nutr. Food Res.* **2012**, *56*, 345−517.

(2) Goldberg, D. M.; Yan, J.; Ng, E.; Diamandis, E. P.; Karumanchiri, A.; Soleas, G.; Waterhouse, A. L. A global survey of *trans*-resveratrol concentrations in commercial wines. *Am. J. Enol. Vitic.* **1995**, *46*, 159−165.

(3) Aggarwal, B. B.; Shishodia, S. Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem. Pharmacol.* **2006**, *71*, 1397−1421.

(4) Delmas, D.; Solary, E.; Latruffe, N. Resveratrol: a phytochemical inducer of multiple cell death pathways: apoptosis, autophagy and mitotic catastrophe. *Curr. Med. Chem.* **2011**, *18*, 1100−1021.

(5) Tili, E.; Michaille, J. J. Resveratrol, microRNAs, inflammation, and cancer. *J. Nucleic Acids* **2011**, *2011*, 102431.

(6) Althar, M.; Back, J. H.; Tang, X.; Kim, K. H.; Kopelovich, L.; Bickers, D. R.; Kim, A. L. Resveratrol: a review of preclinical studies for human cancer prevention. *Toxicol. Appl. Pharmacol.* **2007**, *224*, 274−283.

(7) Bishayee, A. Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials. *Cancer Prev. Res. (Phila.).* **2009**, *2*, 409−418.

(8) Renaud, S.; de Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

(9) Jeandet, P.; Douillet-Breuil, A. C.; Bessis, R.; Debord, S.; Sbaghi, M.; Adrian, M. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *J. Agric. Food Chem.* **2002**, *50*, 2731−2741.

(10) Ribeiro de Lima, M. T.; Waffo-Tegui, P.; Teissedre, P.-L.; Pujolas, A.; Vercauteren, J.; Cabanis, J.-C.; Merillon, J.-M. Determination of stilbenes (*trans*-astringin, *cis*- and *trans*-piceid and *cis*- and *trans*- resveratrol) in Portuguese wines. *J. Agric. Food Chem.* **1999**, *47*, 2666−2670.

(11) Ohyama, M.; Tanaka, T.; Ito, T.; Iinuma, M.; Bastow, K. F.; Lee, K. H. Antitumor agents 200. Cytotoxicity of naturally occurring resveratrol oligomers and their acetate derivatives. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3057−3060.

(12) Colin, D.; Limagne, E.; Jeanningros, S.; Jacquel, A.; Lizard, G.; Athias, A.; Gambert, P.; Hichami, A.; Latruffe, N.; Solary, E.; Delmas, D. Endocytosis of resveratrol via lipid rafts and activation of downstream signaling pathways in cancer cells. *Cancer Prev. Res. (Phila.).* **2011**, *4*, 1095−1106.

(13) Delmas, D.; Passilly-Degrace, P.; Jannin, B.; Malki, M. C.; Latruffe, N. Resveratrol, a chemopreventive agent, disrupts the cell cycle control of human SW480 colorectal tumor cells. *Int. J. Mol. Med.* **2002**, *10*, 193−199.

(14) Timmers, S.; Konings, E.; Bilet, L.; Houtkooper, R. H.; van de Weijer, T.; Goossens, G. H.; Hoeks, J.; van der Krieken, S.; Ryu, D.; Kersten, S.; Moonen-Kornips, E.; Hesselink, M. K.; Kunz, I.; Schrauwen-Hinderling, V. B.; Blaak, E. E.; Auwerx, J.; Schrauwen, P. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab.* **2011**, *14*, 612−622.

(15) Brown, V. A.; Patel, K. R.; Viskaduraki, M.; Crowell, J. A.; Perloff, M.; Booth, T. D.; Vasilinin, G.; Sen, A.; Schinas, A. M.; Piccirilli, G.; Brown, K.; Steward, W. P.; Gescher, A. J.; Brenner, D. E. Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. *Cancer Res.* **2010**, *70*, 9003−9011.

(16) Patel, K. R.; Brown, V. A.; Jones, D. J.; Britton, R. G.; Hemingway, D.; Miller, A. S.; West, K. P.; Booth, T. D.; Perloff, M.; Crowell, J. A.; Brenner, D. E.; Steward, W. P.; Gescher, A. J.; Brown, K. Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. *Cancer Res.* **2010**, *70*, 7392−7399.

(17) Howells, L. M.; Berry, D. P.; Elliott, P. J.; Jacobson, E. W.; Hoffmann, E.; Hegarty, B.; Brown, K.; Steward, W. P.; Gescher, A. J. Phase I randomized, double-blind pilot study of micronized resveratrol (SRT501) in patients with hepatic metastases − safety, pharmacokinetics, and pharmacodynamics. *Cancer Prev. Res. (Phila.).* **2011**, *4*, 1419−1425.

(18) Patel, K. R.; Scott, E.; Brown, V. A.; Gescher, A. J.; Steward, W. P.; Brown, K. Clinical trials of resveratrol. *Ann. N.Y. Acad. Sci.* **2011**, *1215*, 161−169.

(19) Calamini, B.; Ratia, K.; Malkowski, M. G.; Cuendet, M.; Pezzuto, J. M.; Santarsiero, B. D.; Mesecar, A. D. Pleiotropic mechanisms facilitated by resveratrol and its metabolites. *Biochem. J.* **2010**, *429*, 273−282.

(20) Lin, H. Y.; Lansing, L.; Merillon, J. M.; Davis, F. B.; Tang, H. Y.; Shih, A.; Vitrac, X.; Krisa, S.; Keating, T.; Cao, H. J.; Bergh, J.; Quackenbush, S.; Davis, P. J. Integrin $\alpha V\beta 3$ contains a receptor site for resveratrol. *FASEB J* **2006**, *10*, 1742−1744.

(21) Bertelli, A.; Bertelli, A. A.; Gozzini, A.; Giovannini, L. Plasma and tissue resveratrol concentrations and pharmacological activity. *Drugs Exp. Clin. Res.* **1998**, *24*, 133−138.

(22) Lançon, A.; Delmas, D.; Osman, H.; Thenot, J. P.; Jannin, B.; Latruffe, N. Human hepatic cell uptake of resveratrol: involvement of both carrier mediated and diffusion process. *Biochem. Biophys. Res. Commun.* **2004**, *316*, 1132−1137.

(23) Hoshino, J.; Park, E. J.; Kondratyuk, T. P.; Marler, L.; Pezzuto, J. M.; van Breemen, R. B.; Mo, S.; Li, Y.; Cushman, M. Selective synthesis and biological evaluation of sulfate-conjugated resveratrol metabolites. *J. Med. Chem.* **2010**, *53*, 5033−5043.

(24) Kondratyuk, T. P.; Park, E. J.; Marler, L. E.; Ahn, S.; Yuan, Y.; Choi, Y.; Yu, R.; van Breemen, R. B.; Sun, B.; Hoshino, J.; Cushman, M.; Jermihov, K. C.; Mesecar, A. D.; Grubbs, C. J.; Pezzuto, J. M. Resveratrol derivatives as promising chemopreventive agents with improved potency and selectivity. *Mol. Nutr. Food Res.* **2011**, *55*, 1249−1265.

(25) Kosaka, N.; Ochiya, T. Unraveling the mystery of cancer by secretory microRNA: horizontal microRNA transfer between living cells. *Front. Genet.* **2012**, *2*, 97.

(26) Grønbaek, M.; Mortensen, E. L.; Mygind, K.; Andersen, A. T.; Becker, U.; Gluud, C.; Sørensen, T. I. Beer, wine, spirits and subjective health. *J. Epidemiol. Community Health* **1999**, *53*, 721−724.

(27) Levi, F.; Pasche, C.; Lucchini, F.; Ghidoni, R.; Ferraroni, M.; La Vecchia, C. Epidemiological evidence that resveratrol from grape is inversely related to breast cancer risk. Study on 369 cases vs 602 controls of Swiss women from 1993 to 2003. *Eur. J. Cancer Prev.* **2005**, *14*, 139−142.

(28) Lee, R. C.; Feinbaum, R. L.; Ambros, V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* **1993**, *75*, 843−866.

(29) Calin, G. A.; Sevignani, C.; Dumitru, C. D.; Hyslop, T.; Noch, E.; Yendamuri, S.; Shimizu, M.; Rattan, S.; Bullrich, F.; Negrini, M.; Croce, C. M. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 2999−3004.

(30) Tili, E.; Michaille, J.-J.; Gandhi, V.; Plunkett, W.; Sampath, D.; Calin, G. A. MiRNAs and their potential for use against cancer and other diseases. *Future Oncol.* **2007**, *3*, 521−537.

(31) Colotta, F.; Allavena, P.; Sica, A.; Garlanda, C.; Mantovani, A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* **2009**, *30*, 1073−1081.

(32) Tili, E.; Michaille, J.-J.; Adair, B.; Alder, H.; Limagne, E.; Taccioli, C.; Volinia, S.; Delmas, D.; Latruffe, N.; Croce, C. M. Resveratrol decreases the levels of miR-155 by upregulating miR-663, a new anti-inflammatory microRNA targeting JunB and JunD. *Carcinogenesis* **2010**, *31*, 1561−1566.

(33) Tili, E.; Michaille, J. J.; Alder, H.; Volinia, S.; Delmas, D; Latruffe, N.; Croce, C. M. Resveratrol modulates the levels of microRNAs targeting genes encoding tumor-suppressors and effectors of TGFβ signaling pathway in SW480 cells. *Biochem. Pharmacol.* **2010**, *80*, 2057−2065.
(34) Bae, S.; Lee, E. M.; Cha, H. J.; Kim, K.; Yoon, Y.; Lee, H.; Kim, J.; Kim, Y. J.; Lee, H. G.; Jeung, H. K; Min, Y. H.; An, S. Resveratrol alters microRNA expression profiles in A549 human non-small cell lung cancer cells. *Mol. Cells* **2011**, *32*, 243−249.
(35) Han, Z.; Yang, Q.; Liu, B.; Wu, J.; Li, Y.; Yang, C.; Jiang, Y. MicroRNA-622 functions as a tumor suppressor by targeting K-Ras and enhancing the anticarcinogenic effect of resveratrol. *Carcinogenesis* **2011**, *33*, 131−139.
(36) Dhar, S.; Hicks, C.; Levenson, A. S. Resveratrol and prostate cancer: promising role for microRNAs. *Mol. Nutr. Food Res.* **2011**, *55*, 1219−1229.
(37) Hagiwara, K.; Kosaka, N.; Yoshioka, Y.; Takahashi, R. U.; Takeshita, F.; Ochiya, T. Stilbene derivatives promote Ago2-dependent tumour-suppressive microRNA activity. *Sci. Rep.* **2012**, *2*, 314.
(38) Yu, Y. -H.; Chen, H.-A.; Chen, P.-S.; Cheng, Y.-J.; Hsu, W.-H.; Chang, Y.-W.; Chen, Y.-H.; Jan, Y.; Hsiao, M.; Chang, T.-Y.; Liu, Y.-H.; Jeng, Y.-M.; Wu, C.-H.; Huang, M.-T.; Su, Y.-H.; Hung, M.-C.; Chien, M.-H.; Chen, C.-Y.; Kuo, M.-L.; Su, J.-L. MiR-520h-mediated FOXC2 regulation is critical for inhibition of lung cancer progression by resveratrol. *Oncogene* **2012**, DOI: 10.1038/onc.2012.74.
(39) Lukiw, W. J.; Zhao, Y.; Cui, J. G. An NF-κB-sensitive micro RNA-146a-mediated inflammatory circuit in Alzheimer disease and in stressed human brain cells. *J. Biol. Chem.* **2008**, *283*, 31315−31322.
(40) Park, S. J.; Ahmad, F.; Philp, A.; Baar, K.; Williams, T.; Luo, H.; Ke, H.; Rehmann, H.; Taussig, R.; Brown, A. L.; Kim, M. K.; Beaven, M. A.; Burgin, A. B.; Manganiello, V.; Chung, J. H. Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases. *Cell* **2012**, *148*, 421−433.
(41) Baarine, M.; Thandapilly, S.; Louis, X.; Mazué, F.; Yu, L.; Delmas, D.; Netticadan, T.; Lizard, G.; Latruffe, N. Pro-apoptotic vs anti-apoptotic properties and cell signaling of dietary resveratrol on normal and on tumour cells. *Genes Nutr.* **2011**, *6*, 161−169.
(42) Gagan, J.; Dey, B. K.; Dutta, A. MicroRNAs regulate and provide robustness to the myogenic transcriptional network. *Curr. Opin. Pharmacol.* **2012**, *12*, 1−6.
(43) Condorelli, G.; Latronico, M. V.; Dorn, G. W., 2nd. microRNAs in heart disease: putative novel therapeutic targets? *Eur. Heart J.* **2010**, *31*, 649−658.
(44) Thum, T.; Gross, C.; Fiedler, J.; Fischer, T.; Kissler, S.; Bussen, M.; Galuppo, P.; Just, S.; Rottbauer, W.; Frantz, S.; Castoldi, M.; Soutschek, J.; Koteliansky, V.; Rosenwald, A.; Basson, M. A.; Licht, J. D.; Pena, J. T.; Rouhanifard, S. H.; Muckenthaler, M. U.; Tuschl, T.; Martin, G. R.; Bauersachs, J.; Engelhardt, S. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* **2008**, *456*, 980−984.
(45) Mukhopadhyay, P.; Mukherjee, S.; Ahsan, K.; Bagchi, A.; Pacher, P.; Das, D. K. Restauration of altered micro RNA expression in the ischemic heart with resveratrol. *PLoS ONE* **2010**, *5*, e15705.
(46) Kaminski, J.; Lançon, A.; Tili, E.; Aires, V.; Demarquoy, J.; Lizard, G.; Michaille, J.-J.; Latruffe, N. Dietary resveratrol modulates metabolic functions in skeletal muscle cells. *J. Food Drug Anal.* **2012**, *20* (Suppl. 1), 398−401.
(47) Chen, J. F.; Mandel, E. M.; Thomson, J. M.; Wu, Q.; Callis, T. E.; Hammond, S. M.; Conlon, F. L.; Wang, D.-Z. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat. Genet.* **2006**, *38*, 228−233.
(48) Howitz, K. T.; Bitterman, K. J.; Cohen, H. Y.; Lamming, D. W.; Lavu, S.; Wood, J. G.; Zipkin, R. E.; Chung, P.; Kisielewski, A.; Zhang, L. L.; Scherer, B.; Sinclair, D. A. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* **2003**, *425*, 191−196.
(49) Knutson, M. D.; Leeuwenburgh, C. Resveratrol and novel potent activators of SIRT1: effects on aging and age-related diseases. *Nutr. Rev.* **2008**, *66*, 591−596.
(50) Tonkin, J.; Villarroya, F.; Puri, P. L.; Vinciguerra, M. SIRT1 signaling as potential modulator of skeletal muscle diseases. *Curr. Opin. Pharmacol.* **2012**, *12*, 1−5.
(51) Zhu, H.; Wu, H.; Liu, X.; Li, B.; Chen, Y.; Ren, X.; Liu, C. G.; Yang, J. M. Regulation of autophagy by a beclin 1-targeted microRNA, miR-30a, in cancer cells. *Autophagy* **2009**, *5*, 816−823.