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Biphasic regulation of spindle assembly checkpoint by low and high concentrations of resveratrol leads to the opposite effect on chromosomal instability

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ABSTRACT

Resveratrol (RSV) is a naturally occurring polyphenolic phytoalexin possessing numerous health-promoting effects. Chromosomal instability (CIN), usually results from defective spindle assembly checkpoint (SAC), is a major contributor to many diseases. While it's recently recognized that RSV exhibits a nonlinear dose response for disease prevention, whether it's the case for its role in CIN remains unknown. Here, we investigated the potential of a broad range of RSV concentrations (0.01–100 μM) on CIN and the underlying mechanisms in human normal colon epithelial NCM460 cells. CIN was measured by cytokinesis-block micronucleus assay; mitotic fidelity was determined by aberrant mitosis analysis; SAC activity was assessed by nocodazole-challenge assay, and the expression of SAC genes was examined by RT-qPCR. We found that 0.1 μM RSV significantly reduced CIN ($P < 0.01$), while 100 μM RSV significantly induced it ($P < 0.05$). Mitotic infidelity was significantly prevented by 0.1 μM RSV but promoted by 100 μM RSV ($P < 0.05$ for both). Moreover, the function of SAC was sustained and impaired by 0.1 μM and 100 μM RSV, respectively. Several SAC genes, including *Aurora-B*, *Aurora-C*, *Plk-1* and *CENP-E*, were significantly up-regulated and down-regulated by 0.1 μM and 100 μM RSV, respectively ($P < 0.05$). In conclusion, RSV exhibited a biphasic dose-dependent effect on CIN that was exerted via the regulation of mitotic fidelity through the SAC network. The health implications of these findings were summarized.

1. Introduction

Resveratrol (RSV) is a polyphenolic phytoalexin synthesized by a variety of plant species (e.g., grapes, apples, raspberries, blueberries, plums, peanuts) in response to injury, UV irradiation and fungal attack. Although both *cis*- (Fig. 1A) and *trans*-isomers (Fig. 1B) of RSV occur in nature, it is generally assumed that the *trans*-form is biologically more active. During the past two decades, a large number of investigations have shown that RSV may slow down the aging process and help prevent age-associated diseases, such as heart disease, cancer, Alzheimer's disease, diabetes, and many others [1]. However, with the recent results from clinical trials and *in vivo* studies, it's shown that low RSV dose retards aging [2], prevents cancer [3] and improves cardiovascular and cerebrovascular function (4,5) more potently than do the higher dose. The therapeutic doses of RSV varied between clinical trials: subjects in ~80% of human clinical trials receiving 5–1000 mg daily [4], resulting in average peak concentrations of blood levels between 0.6 and 137 μM [3]. High doses RSV supplementation has been reported to possess adverse gastrointestinal effect in humans [5]. These results indicate that diverse beneficial effects induced by RSV are strictly dose-dependent:

Abbreviations: APC/C, anaphase-promoting complex/cyclosome; BNC, binucleated cell; CB, chromatin bridge; CBMN, cytokinesis-block micronucleus assay; CIN, chromosomal instability; CL, chromosome lagging; CMA, chromosome misalignment; MN, micronucleus; MPA, multipolar alignment; MPS, multipolar segregation; NB, nuclear bud; NPB, nucleaoplasmic bridge; Noc, nocodazole; RSV, resveratrol; SAC, spindle assembly checkpoint

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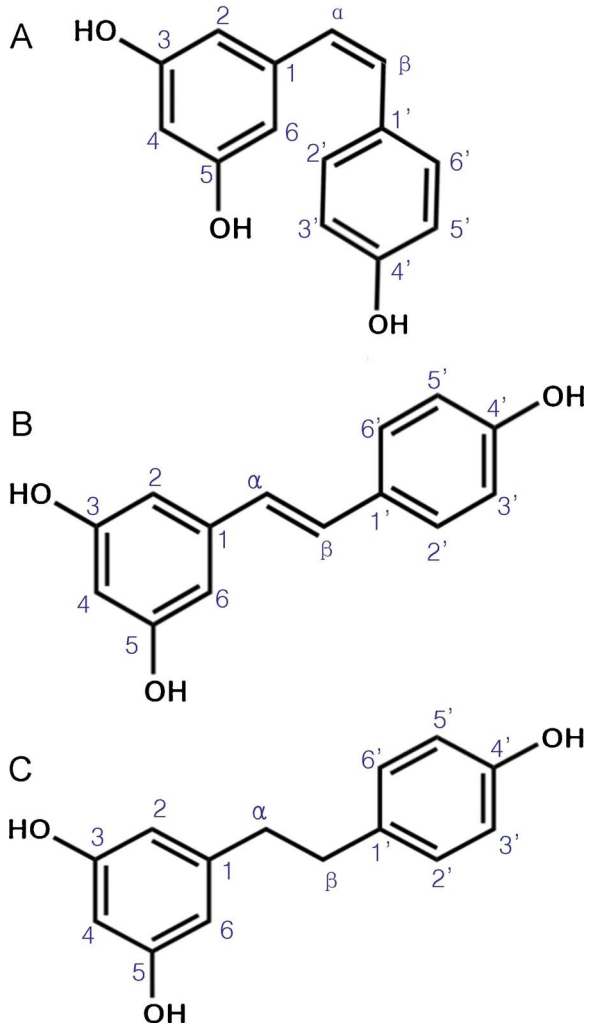

Fig. 1. Chemical structures of resveratrol (3,5,4′-trihydroxystilbene, shown in A and B are the *cis*- and *trans*-conformation, respectively) and dihydroresveratrol (*trans*-3,5,4′-trihydroxybibenzyl, C).

whereas low doses of RSV have been implicated in pronounced health-promoting effects, high doses of RSV promote unwanted side effects. In line with this, RSV often displays a biphasic dose response [6] with quantitative features consistent with the hormetic dose response, a phenomenon that is characterized by a low-dose stimulation and a high-dose inhibition [7,8].

Chromosomal instability (CIN) is defined as an increase in the rate at which whole chromosomes or large chromosomal fragments are gained or lost. CIN has long been known to be the underlying cause of human aging [9] and many aging-related diseases such as cancer [10] and Alzheimer's disease [11]. One way protects cells against CIN is through the accurately control of chromosome segregation during mitosis. To this end, the spindle assembly checkpoint (SAC) regulates the proper attachment of microtubules to kinetochores and the tension between the kinetochores of sister chromatids, and blocks progression to anaphase until each chromosome is correctly attached to the spindle and under bipolar tension. Therefore, SAC is a molecular safeguard mechanism that preserves the chromosomes from structural and numerical alterations and protects cells from the direct consequences following them [12]. Dysregulation of the expression of some key SAC genes, such as *CENP-E*, *CENP-A*, *CENP-F* and *BubR1*, contributes to the increased CIN in aging and cancers cells [13,14]. In contrast, transgenic mice overexpressing *BubR1* exhibits an increased protection against CIN and cancer, as well as extended healthy lifespan [14], indicating that protection against CIN is a promising way to delay aging and prevent aging-related diseases.

Given the roles of RSV in delaying aging and preventing aging associated diseases, the aims of the present study were to investigate the effect of RSV on CIN and to determine if these results can be explained through modulating the SAC. In light of the hormetic dose-response nature of RSV [6], it is conceivable that RSV at low doses may reduce spontaneous CIN, whereas at high dose may induce CIN in human normal cells. To examine this hypothesis, we selected human normal colon mucosal epithelial cell line NCM460 as an appropriate *in vitro* mode, for two reasons: (i) the gastrointestinal tract is the direct target organ of RSV [3,5] and (ii) NCM460 cells were proved to be sensitive to chemicals that protect against or induce CIN [15–17].

We firstly explored the effect of a broad range of RSV concentrations on the CIN level of NCM460 cells using the cytokinesis-block micronucleus (CBMN) assay. Secondly, we investigated the effect of RSV on chromosome alignment and segregation using aberrant mitosis analysis. Finally, we determined the SAC activity using nocodazole (Noc) challenge assay and measured the transcriptional expression of several SAC genes using RT-qPCR. Our analysis revealed that RSV exhibited a biphasic effect on CIN, and we further found this effect was exerted via the comprehensive regulation of mitotic fidelity through the SAC pathway.

2. Materials and methods

2.1. Chemicals

Trans-RSV (purity ≥ 99%) obtained from Cayman Chemical (Ann Arbor, MI, USA), and cytochalasin-B and Noc obtained from Sigma-Aldrich (St.Louis, MO, USA). Stock solution of RSV (43.85 mM), cytochalasin-B (600 μg/mL) and Noc (4 mg/mL) were prepared in dimethyl sulfoxide (DMSO). The solution was stored at −20 °C and diluted to the desired concentration in medium immediately before use. The final concentration of DMSO was never exceeded 0.25% (*v/v*), this concentration did not exert any cytotoxic and genotoxic effects.

2.2. Cell culture

NCM460 (an adherent cell line) was obtained from INCELL (San Antonio, TX, USA) and maintained as a monolayer in 75 cm^2 flasks (Corning, NY, USA) in RPMI 1640 medium (Gibco, NY, USA) supplemented with 10% fetal bovine serum (Gibco), 1% penicillin [5000 IU/mL]/streptomycin [5 mg/mL] solution (Gibco), 1% L-glutamine (2 mM) (Sigma), and kept at 37 °C in a 5% CO_2 environment. In order to ensure that endogenous CIN had not occurred significantly, NCM460 cells at early passages (ranging from P15 to P25) were used for this study.

2.3. Trypan blue exclusion assay

The exclusion of Trypan blue dyes is often used as an indication of membrane integrity of living cells, as the dyes can cross the compromised cell membrane and stain cellular targets or structures in dead cells. NCM460 cells were seeded into 24-well plates (Corning, NY, USA) at a density of 1×10^5 cells/mL and exposed to different concentrations of RSV (0, 0.01, 0.1, 1, 10, 100 μM). After 24 h incubation, adherent and non-adherent cells were detached from plates and collected. Cells were incubated with trypan blue to exclude dead cells, and counted with a hemacytometer. This procedure repeated three times in duplicate for each RSV concentration.

2.4. Clonogenic survival assay

The clonogenic survival assay was used to assess cellular sensitivity to cytotoxic treatments as it tests the fundamental aspect of survival, a cell's ability to undergo sufficient proliferation so as to form a colony. After 24 h treatment of RSV, cells were seeded at a density of 1000

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