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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A B S T R A C T

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 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 \textit{cis}- (Fig. 1A) and \textit{trans}-isomers (Fig. 1B) of RSV occur in nature, it is generally assumed that the \textit{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 \textit{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 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, nucleoplasmic bridge; Noc, nocodazole; RSV, resveratrol; SAC, spindle assembly checkpoint

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protection against CIN is a promising way to delay aging and prevent and cancer, as well as extended healthy lifespan[14], indicating that increased CIN in aging and cancers cells[13,14]. In contrast, transgenic following them[12]. Dysregulation of the expression of some key SAC mechanism that preserves the chromosomes from structural and nu-
merical alterations and protects cells from the direct consequences 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 micro-nucleus (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 bi-
phasic 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 INCCELL (San Antonio, TX, USA) and maintained as a monolayer in 75 cm² 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% CO2 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 × 10⁵ 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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*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).*
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