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Resveratrol induces DNA damage in colon cancer cells by poisoning topoisomerase II and activates the ATM kinase to trigger p53-dependent apoptosis

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ABSTRACT

Resveratrol (trans-3,4′,5-trihydroxystilbene) is a natural polyphenol synthesized by various plants such as grape vine. Resveratrol (RSV) is a widely studied molecule, largely for its chemopreventive effect in different mouse cancer models. We propose a mechanism underlying the cytotoxic activity of RSV on colon cancer cells. Our data show that resveratrol induces apoptosis, as observed by the cleavage of PARP-1 and chromatin condensation. We show that the tumor suppressor p53 is activated in response to RSV and participates to the apoptotic process. Additionally, we show that HCT-116 p53 wt colon carcinoma cells are significantly more sensitive than HCT-116 p53−/− cells to RSV. RSV induces DNA damage including double strand breaks, as evidenced by the presence of multiple γ-H2AX foci in 50% of cells after a 24 h treatment with 25 μM RSV. The formation of DNA damage does not appear to rely on a pro-oxidant effect of the molecule, inhibition of topoisomerase I, or DNA intercalation. Rather, we show that DNA damage is the consequence of type II topoisomerase poisoning. Exposure of HCT-116 cells to RSV leads to activation of the Ataxia Telangiectasia Mutated (ATM) kinase, and ATM is required to activate p53.

1. Introduction

Resveratrol is a natural polyphenol found in the skin of various fruits including grapes and berries (Pervaiz and Holme, 2009). A first study published in 1997 described that the molecule prevents the development of pre-neoplastic lesions in cultured carcinogen-treated mammary glands and exerts protection against skin cancer in a mouse model (Jang et al., 1997). Since then, resveratrol (RSV) has been widely studied for this now fairly well evidenced chemopreventive properties. In addition, a potential use of RSV or derivatives in cancer chemotherapy is investigated. Indeed, reduction of tumor growth has been observed in different mouse cancer models including neuroblastoma, colon, prostate, liver and breast cancer (Carter et al., 2014; Bishayee, 2009; Athar et al., 2007). RSV acts as a suppressor of inflammatory processes that can influence cancer progression (Shakibaei et al., 2009). The activity of RSV is also linked to its ability to inhibit tumor invasion, metastasis formation as well as neo-angiogenesis. Finally, other properties of RSV relevant to cancer prevention or therapy include modulation of cell redox status, inhibition of cell proliferation and induction of apoptosis (Athar et al., 2007; Shakibaei et al., 2009; Vang et al., 2011; Fulda, 2010).

Among various mechanisms proposed to explain the apoptosis inducing properties of RSV on cancer cells, the molecule is known for its modulation of BCL-2 family members expression. RSV is able to upregulate the expression of pro-apoptotic proteins such as BAX, BAK, PUMA and NOXA while decreasing that of anti-apoptotic members like BCL-2, MCL1 and BCL-XL. Therefore, RSV could favor outer mitochondrial membrane (OMM) permeabilization and release of the transmembrane apoptosis effectors in the cytosol (Shankar et al., 2007; Park et al., 2001). RSV also modulates the

Abbreviations: ab, antibody; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and rad3 related; DAPI, 4′,6-diamidino-2-phenylindole; DNA-PK, DNA-dependent protein kinase; DOX, doxorubicin; ERK1/2, extracellular signal-regulated kinase 1/2; H2AX, histone H2A variant H2AX; HPV, human papillomavirus; kDNA, kinetoplast DNA; KO, knockout; MDM2, mouse double minute 2; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide; NAC, N-acetyl cysteine; NUT, nutlin-3a; p38MAPK, p38 mitogen-activated protein kinase; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; RSV, resveratrol; SA-β-gal, senescence-associated beta-galactosidase; Ser, serine; TOPO, topoisomerase; wt, wild type.

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extrinsic pathway of apoptosis that relies on binding of ligands such as TNF-α, FASL or TRAIL on their specific membrane receptor. RSV drives redistribution of the receptor FAS/CD95 to lipid rafts, therefore facilitating activation of this pathway. It also sensitizes cells to TRAIL-induced apoptosis presumably through similar mechanisms (Athar et al., 2007).

The tumor suppressor p53 also appears to be a target of RSV since the molecule is able to activate p53 in a variety of cancer cell lines including breast, colon and prostate cancer cells as well as osteosarcoma and B-cell lymphoma (Athar et al., 2007). However, how RSV signals to p53 is unknown. Alteration of pathways regulating the apoptotic process and direct mutation of genes involved in apoptosis is a hallmark of cancer (Hanahan and Weinberg, 2011). Central to apoptosis, the tumor suppressor p53 is the most frequently mutated gene in human cancer, with an overall mutation rate over 50% (Brown et al., 2009; Toledo and Wahl, 2006; Donehower and Lozano, 2009; Brosh and Rotter, 2009). As a transcription factor, p53 induces or represses the expression of a variety of genes which products have respectively a pro-apoptotic (BAX, NOXA, PUMA, KILLER/DR5, FAS/CD95, ...) or a pro-survival role (BCL-2, SURVIVIN, MDR1, ...) (Laptenko and Prives, 2006; Vousden and Lane, 2007). Additionally, p53 can trigger apoptosis by acting directly at the mitochondria. Indeed, in response to an apoptotic insult, p53 translocates to mitochondria and induces OMM permeabilization. This activity requires direct interaction of p53 with BCL-2 family members, and importantly BAK (Marchenko et al., 2000; Dumont et al., 2003; Mihara et al., 2003; Leu et al., 2004; Chipuk et al., 2004; Pietsch et al., 2008).

The aim of this study is to investigate the mechanism(s) of RSV-induced cell death in colon cancer cells, and the involvement of p53 in this process. We show that RSV is able to induce extensive DNA damage and more specifically DNA double strand breaks in human colon carcinoma cells, leading to p53-dependent apoptosis through the activation of the Ataxia Telangiectasia Mutated (ATM) kinase. We also provide evidence that DNA damage upon RSV treatment is due to topoisomerase II poisoning, and not an elevation of the intracellular level of reactive oxygen species (ROS).

2. Results

2.1. Resveratrol induced apoptosis is enhanced in the presence of wt p53

Cultures of HCT-116 p53 wt colon carcinoma cells were exposed, for 0, 24 or 48 h, to increasing concentrations of RSV and labeled with DAPI at the end of treatments in order to measure chromatin condensation, a morphological characteristic of the apoptotic nucleus. Following this treatment, the percentage of cells containing condensed chromatin increased in a time- and dose-dependent manner (Fig. 1A). PARP cleavage was also clearly observed in cultures incubated for 24 h to concentrations of RSV equal or above 50 μM (Fig. 1B). In addition, RSV triggered a marked induction of the tumor suppressor p53 (Fig. 1C) as well as its translocation to the mitochondria, concomitant to cytochrome c release (Data not shown). We next examined whether RSV induced cell death was dependent on p53 function. HCT-116 p53 wt cells and their somatic-cell knock-out derivative HCT-116 p53−/− (Bunz et al., 1999) were incubated for 24 h in the presence of 50 μM RSV and cell viability was thereafter quantified by flow cytometry (co-labeling with LDS751 and propidium iodide). Data show that the percentage of death is significantly higher in p53 wt cell populations (38.4 ± 5.1%) as compared to their null derivative (21.9 ± 4.8%) ($p < 0.05$, Fig. 1D). PARP cleavage was also assessed by western blotting in HCT-116 p53 wt and p53−/− cells exposed to different concentrations of RSV for 24 h. A marked induction of p53 was observed in wt cells. Moreover, densitometry analysis of the bands revealed that PARP cleavage was 2-fold more abundant in wt cells, indicating that presence of the tumor suppressor indeed enhances apoptosis (Fig. 1E). A similar dependence of cell death on p53 function was observed using the PA1 ovarian teratocarcinoma cell line (p53 wt) and its derivative expressing the human papillomavirus (HPV) E6 protein (Fig. S1).

2.2. Resveratrol induces formation of multiple γ-H2AX foci

RSV was previously shown to damage plasmid DNA in vitro in the presence of copper cations, by an oxidative mechanism that requires generation of peroxides (Fukuhara and Miyata, 1998; Fukuhara et al., 2006). We therefore sought to determine whether RSV is able to induce DNA damage in HCT-116 colon carcinoma cells and whether this is dependent on the generation of reactive oxygen species (ROS). At first, we monitored the formation of γ-H2AX foci. In response to DNA damage and more specifically DNA strand breaks, ATM and other kinases such as ATR and DNA-PK are known to phosphorylate the histone variant H2AX on Ser-139 (γ-H2AX) (Scully and Xie, 2013; Valdiglesias et al., 2013). Very few cells presented with γ-H2AX foci in untreated controls (2.9 ± 3.4%) or after a 24 h exposure to the MDM2 inhibitor nutlin-3a (NUT) (0.3 ± 0.4%), as expected (Fig. 2A). Treatment of cells with doxorubicin (DOX) at 3 μM for 24 h triggered formation of γ-H2AX foci (51.7 ± 10%), in accordance with its described mechanism of action (Yang et al., 2014). Interestingly, we found a high proportion of γ-H2AX positive cells in the presence of RSV (Fig. 2A). Formation of γ-H2AX foci was directly proportional to the concentration of the polyphenol, with 50% of cells displaying multiple foci after a 24 h treatment in the presence of 25 μM RSV (Fig. 2B).

2.3. Formation of γ-H2AX foci and cell death are not inhibited by the anti-oxidant N-acetyl cysteine

As a polyphenol, RSV displays anti-oxidant properties (Gusman et al., 2001; Pervaiz and Holme, 2009). However, some reports also suggest a pro-oxidant action of RSV. For instance, chronic exposure of HCT-116 cells to RSV was reported to induce senescence and this was associated to an elevation of the intracellular level of reactive oxygen species (ROS), including hydrogen peroxide and superoxide anion (Heiss et al., 2007). To determine whether the cell death and γ-H2AX foci were the result of pro-oxidant activity of RSV, we analyzed the impact of the anti-oxidant N-acetyl cysteine (NAC). We found that NAC is consistently unable to inhibit formation of γ-H2AX foci in HCT-116 cells treated for 24 h in the presence of RSV (Fig. 2C). We also tested whether NAC was able to improve the viability of cell populations exposed to RSV. HCT116 cells were incubated for 72 h to increasing concentrations of RSV (from 0 to 500 μM) in the presence or not of 5 mM NAC. We found that cell viability, as determined by MTT assays, was not rescued by NAC (Fig. 2D). In contrast, NAC was highly protective against hydrogen peroxide, thus verifying the efficiency of the antioxidant (Fig. 2D). The combined data support the premise that RSV-induced cell death is not due to its pro-oxidant function.

2.4. Resveratrol is not a DNA intercalating agent and does not inhibit topoisomerase I

To determine the mechanism by which RSV induces DNA damage, we next analyzed whether RSV acts as a DNA intercalating agent or affects the activity of topoisomerases (TOPO). We first performed DNA unwinding tests in the presence of TOPO I, an enzyme able to negatively supercoil a relaxed circular DNA plasmid in the presence of an intercalating agent (Van Quaquebeke