

THE POLYPHENOL RESVERATROL ALTERS
SPHINGOLIPID METABOLITES IN SW480 COLORECTAL
CARCINOMA CELLS IN A SPECIFIC WAY

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Abstract

Resveratrol is a naturally occurring polyphenol exhibiting various beneficial effects, such as anti-tumour, antioxidant, anti-inflammatory, and neuroprotective effects. The anti-cancer activity of resveratrol is related to alterations in specific sphingolipid metabolites. In this work we used SW480 colorectal carcinoma cells to analyze the effect of resveratrol on key sphingolipids, as well as on sphingolipid-metabolizing enzymes. Our findings show that resveratrol treatment of colorectal tumour cells, unlike other tumour cells, induces elevation in the main pro-apoptotic factor ceramide, whereas no significant changes were observed in the proliferation-supporting sphingolipid sphingosine-1 phosphate as well as in the enzyme maintaining its level. The presented data will contribute to a better understanding of the effect of phytoalexins on the regulation of sphingolipid metabolism and may serve as a basis for developing complex anti-cancer therapeutic approaches involving natural antitumour and antioxidant agents, such as polyphenols.

Key words: resveratrol, sphingomyelin, ceramide, sphingosine-1-phosphate, SW480 cells

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Introduction. Resveratrol is a widely investigated phytoalexin, which is associated with various beneficial effects such as antioxidant, anti-neoplastic and anti-ageing, among others [1–5]. A large number of investigations are devoted to the impact of resveratrol on different pathological conditions such as oxidative stress, age-related diseases, neurodegenerative disorders, inflammation, neoplastic processes, etc. [3].

Resveratrol has been found to affect sphingolipid metabolism in various cell types, including cancer cells. Sphingolipids are functionally active lipid molecules, their activity being related to essential processes like differentiation, proliferation and apoptosis [6–10]. In our previous studies, we reported that treatment of A549 lung adenocarcinoma cells with resveratrol induces reduction of sphingomyelin and elevation of ceramide level, the latter being associated with apoptotic processes [11]. We also reported that resveratrol induces increase in the activity and expression of neutral sphingomyelinase, leading to accumulation of ceramide in these cells. The levels of the functionally active sphingolipids sphingosine and sphingosine-1-phosphate were significantly altered due to resveratrol treatment. This observation holds significance, particularly due to sphingosine-1-phosphate's crucial role in promoting cell proliferation and survival. Its decrease carries notable implications, especially in the context of cancer cells. We also reported similar changes in the sphingolipid metabolism in ras-transformed 3T3 fibroblasts [12]. In these cells, resveratrol also induces a decrease in ceramidase activity, which additionally supports the accumulation of the pro-apoptotic ceramide.

In spite of the vast number of studies related to the influence of resveratrol on the lipid metabolism of cancer cells, the mechanism of this effect on the main functionally active sphingolipid metabolites still needs further clarification.

The purpose of the present study was to examine how resveratrol affects the primary sphingolipid pathway in human colorectal adenocarcinoma SW480 cells, exploring its potential to induce apoptotic processes and limit the production of pro-survival sphingolipid metabolites.

Materials and methods. Resveratrol was purchased from Sigma-Aldrich. Treatment with resveratrol was performed as explained elsewhere [12]. In brief, SW480 cells (5×10^5 /well) were seeded in a 24-well plate overnight and treated with 80 μ M of resveratrol for 24 h. This concentration of resveratrol was used because the measured alterations in sphingomyelin levels were linear up to 120 μ M. Resveratrol was delivered from a stock solution in dimethyl sulphoxide. Lipid extraction was performed according to BLIGH and DYER [13]. Phospholipids were determined according to the procedure of KAHOVCOVÁ and ODAVIĆ [14]. Neutral sphingomyelinase activity was measured by the Neutral Sphingomyelinase Activity assay kit (Echelon Biosciences, Cat.# K-1800). Ceramidase activity was estimated by measuring the released sphingosine from ceramide as described elsewhere [11]. Sphingosine-1 phosphate level and sphingosine kinase activity were determined using ELISA kits from Abcam according to the manufacturer's in-

structions. The expression levels of neutral sphingomyelinase and sphingosine kinase were measured as described by MOMCHILOVA et al. [11].

Statistical processing of the data was performed with the unpaired *t*-test, using In Stat software (Graph Pad In Stat 3.1, developed by Graph Pad Software, San Diego, CA, USA).

Results. We studied the effect of resveratrol on the major sphingolipids, sphingomyelin (SM), ceramide, sphingosine and sphingosine-1 phosphate in SW480 cells (Fig. 1). The results showed that the level of SM was not significantly altered, whereas the content of one of the most physiologically active sphingolipids, ceramide, which is basically related to apoptotic processes, was markedly increased (Fig. 1A, B).

The presented results show that resveratrol affects predominantly the level of the pro-apoptotic lipid ceramide and has no effect on the most abundant sphingolipid – sphingomyelin. In an attempt to analyze the biochemical mechanism underlying the reported observations, we studied the activity and the level of

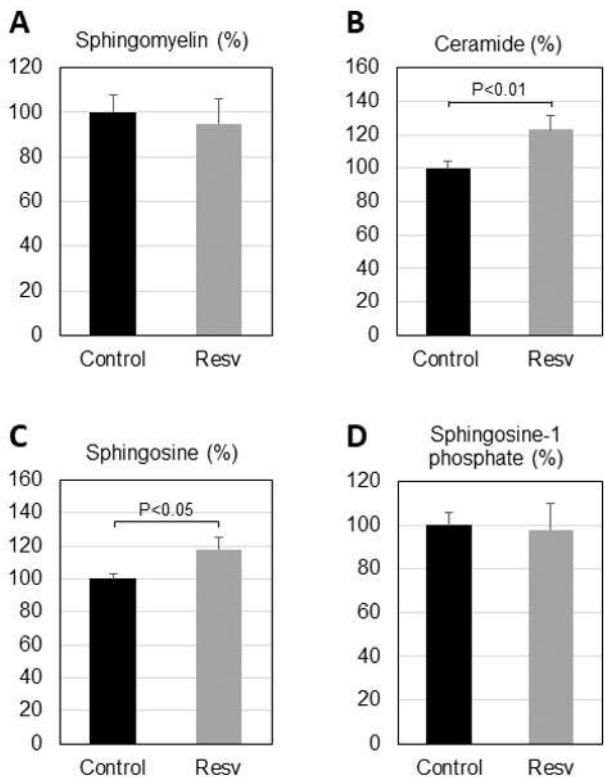


Fig. 1. Changes in sphingomyelin (A), ceramide (B), sphingosine (C), and sphingosine-1 phosphate (D) levels in control and resveratrol (Resv)-treated SW480 cells. Results are presented as percentages relative to control (100%) values. Only ceramide and sphingosine levels demonstrated statistically significant alterations compared to the controls

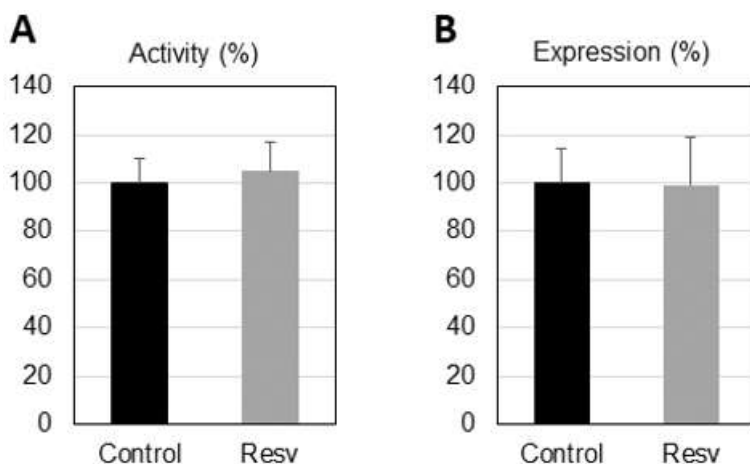


Fig. 2. Changes in the specific activity (A) and the level of expression (B) of neutral sphingomyelinase in control and resveratrol (Resv)-treated SW480 cells. Results are presented as percentage of control cells (100%). The observed differences were not statistically significant

expression of the main SM-hydrolyzing and ceramide-producing enzyme, neutral sphingomyelinase (Fig. 2).

The obtained data showed that neither the specific activity, nor the expression of neutral sphingomyelinase were altered by resveratrol treatment of SW480 cells implying that the elevated content of ceramide was not due to changes in the major sphingomyelin-degrading and ceramide-producing enzyme, neutral sphingomyelinase.

Further, we studied the impact of resveratrol on the other two enzymes responsible for maintenance of the level of ceramide: ceramidase and ceramide synthase. The experimental data demonstrated that the enzyme performing ceramide degradation – ceramidase, was down-regulated as a result of resveratrol treatment, whereas the activity of ceramide synthase was not significantly affected (Fig. 3).

Sphingosine and particularly sphingosine-1 phosphate are sphingolipids with particular physiological significance, which is why we analyzed the alterations in their levels, induced by resveratrol treatment (Fig. 1C, D). Especially sphingosine-1 phosphate is associated with cell proliferation and survival, which underlies its specific physiological significance. The obtained results showed that the level of sphingosine was elevated by about 20%, but sphingosine-1 phosphate was not affected significantly by resveratrol treatment. The result showing the lack of effect of resveratrol on the content of sphingosine-1 phosphate was rather unexpected, because in most of the tumour cells treated with resveratrol and other polyphenols a pronounced reduction of this sphingolipid is observed.

The key enzyme that controls the balance between sphingosine and sphingosine-1 phosphate is sphingosine kinase 1, which supports phosphorylation of sphingosine. Alterations in sphingosine kinase 1 regulation result in changes in the

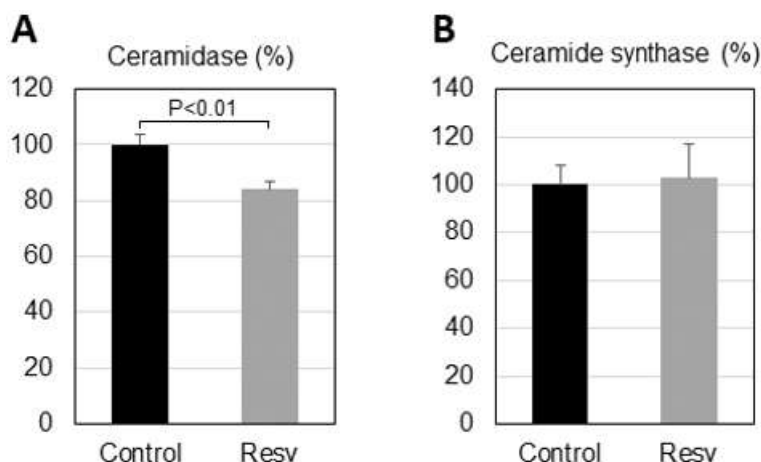


Fig. 3. Alterations in the activity of two sphingolipid-metabolizing enzymes: ceramidase (A) and ceramide synthase (B) in resveratrol-treated SW480 cells. The results are presented as % of control values. Only the changes in ceramidase activity were statistically significant compared to the controls

levels of the pro-proliferative lipid sphingosine-1 phosphate, which is accompanied by changes in the balance between apoptosis and survival. Analysis of the activity and expression of sphingosine kinase 1 in resveratrol-treated SW480 cells showed no statistically significant changes in both investigated parameters (Fig. 4).

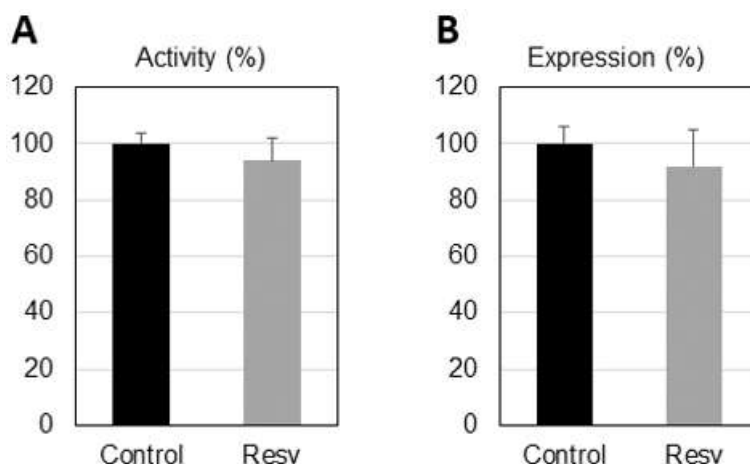


Fig. 4. Alterations in the activity (A) and expression level (B) of sphingosine kinase 1 were investigated in non-treated (Control) or resveratrol (Resv)-treated SW480 tumour cells. The results are displayed as percentages relative to control cells set at 100%. Notably, the observed variances did not reach statistical significance

Discussion. Resveratrol is a naturally occurring phytoalexin that has been reported to exhibit antioxidant, anti-inflammatory and anti-cancer effect on cells [4,

5, 15, 16]. In the present study we used as experimental model colorectal adenocarcinoma SW480 cells to analyze the effect of resveratrol on major functionally active components of the sphingolipid pathways. Since colorectal cancer is one of the most widely spread types of malignancies, we explored these cells as a model to investigate the possibilities of natural antioxidants to affect the sphingolipid metabolism, the latter being known as a potent regulator of the balance between apoptosis and survival of cells.

The obtained results showed that the level of the major sphingolipid sphingomyelin was not altered, whereas the content of ceramide was elevated as a result of resveratrol treatment of SW480 cells (Fig. 1A, B). In addition, the specific activity and expression level of neutral sphingomyelinase, the primary enzyme responsible for sphingomyelin hydrolysis and ceramide production, exhibited minimal change in cells incubated with resveratrol (Fig. 2). In our previous studies, we analyzed the influence of resveratrol on the level of cellular sphingolipids as a result of treatment of A549 lung adenocarcinoma cells [6]. We reported a decrease of sphingomyelin and an increase of ceramide, but both the activity of neutral sphingomyelinase and its expression were elevated. Thus, it is likely that although the effect of resveratrol on the level of ceramide in cancer cells seems to be similar, the mechanisms underlying ceramide accumulation occur to be different. In A549 cells ceramide level was augmented predominantly as a result of up-regulation of the key sphingolipid-metabolizing enzyme neutral sphingomyelinase, whereas in SW480 cells ceramide elevation was mainly due to down-regulation of the main ceramide-degrading enzyme, ceramidase (Fig. 3). This is an interesting finding because the differences in the mechanism underlying the observed alterations suggest differences in the possible therapeutic approaches, that should be developed in agreement with the specificities of the corresponding cancer cells.

Since the sphingolipid pathways play a crucial role in regulation of the balance between proliferation and apoptosis [6], it is quite likely, that one of the major mechanisms, underlying the effect of resveratrol on tumour cells is its impact on the sphingolipid-metabolizing enzymes.

It should be pointed out that ceramidase activity, which contributes for maintenance of ceramide levels, was down regulated in resveratrol-treated SW480 cells, implying that the observed elevation of ceramide level was a result predominantly of this enzyme activity (Fig. 3). Thus, of all three enzymes responsible for maintenance of ceramide level, sphingomyelinase, ceramidase and ceramide synthase, the only enzyme affected by resveratrol treatment of SW480 cells was ceramidase. However, the level of expression of this enzyme remained almost unchanged in the cells incubated with resveratrol (data not shown). In our previous studies with resveratrol-treated A549 cells we reported elevation of both the specific activity and a slight down-regulation of the expression of ceramidase [11].

The level of sphingosine and especially of sphingosine-1 phosphate is associated with cellular proliferation and survival. The presented results show that

sphingosine level was augmented, whereas sphingosine-1 phosphate was not altered as a result of resveratrol treatment (Fig. 4). The enzyme producing sphingosine-1 phosphate through phosphorylation of sphingosine is sphingosine-kinase 1 and it is essential for maintenance of the balance between the pro-apoptotic ceramide and the pro-proliferative factor sphingosine-1 phosphate. In the case with resveratrol-treated SW480 cells sphingosine-kinase activity and expression remained unchanged compared to untreated cells (Fig. 4). Interestingly, studies on A549 cells treated with resveratrol revealed down-regulation of both the activity and expression of sphingosine kinase, which was in good agreement with the reported decrease of the pro-survival sphingolipid agent, sphingosine-1 phosphate. Sphingosine kinase is the main regulatory enzyme in the so-called “sphingolipid rheostat” and, as mentioned above, it plays an essential role in the balance between the processes of apoptosis and proliferation. Our findings show that resveratrol treatment of colorectal tumour cells induces elevation in the main pro-apoptotic factor ceramide, whereas no significant changes were observed in the proliferation-supporting sphingolipid, sphingosine-1 phosphate as well as in the enzyme maintaining its level.

In conclusion, the presented results demonstrate that treatment of colorectal carcinoma cells with the phytoalexin resveratrol stimulates the processes of programmed cell death through ceramide elevation, but it does not contribute to restriction of the proliferative processes, unlike for example resveratrol, when applied to oncogene-transformed cells [12]. The presented data shed some light on the effect of polyphenols on the regulation of sphingolipid metabolism and could be useful in the development of complex antitumour therapeutic approaches, involving natural antitumour agents.

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