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Research Article

Resveratrol upregulates Egr-1 expression and activity involving extracellular signal-regulated protein kinase and ternary complex factors

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ABSTRACT

Many intracellular functions have been attributed to resveratrol, a polyphenolic phytoalexin found in grapes and in other plants. Here, we show that resveratrol induces the expression of the transcription factor Egr-1 in human embryonic kidney cells. Using a chromosomally embedded Egr-1-responsive reporter gene, we show that the Egr-1 activity was significantly elevated in resveratrol-treated cells, indicating that the newly synthesized Egr-1 protein was biologically active. Stimulus-transcription coupling leading to the resveratrol-induced upregulation of Egr-1 expression and activity requires the protein kinases Raf and extracellular signal-regulated protein kinase ERK, while MAP kinase phosphatase-1 functions as a nuclear shut-off device that interrupts the signaling cascade connecting resveratrol stimulation with enhanced Egr-1 expression. On the transcriptional level, Elk-1, a key transcriptional regulator of serum response element-driven gene transcription, connects the intracellular signaling cascade elicited by resveratrol with transcription of the Egr-1 gene. These data were corroborated by the observation that stimulation of the cells with resveratrol increased the transcriptional activation potential of Elk-1. The SRE as well as the GC-rich DNA binding site of Egr-1 function as resveratrol-responsive elements. Thus, resveratrol regulates gene transcription via activation of the stimulus-regulated protein kinases Raf and ERK and the stimulus-responsive transcription factors TCF and Egr-1.

Introduction

The polyphenol resveratrol (trans-3,4′,5-trihydroxystilbene) is an abundant constituent of the human diet, as resveratrol is found in a large number of plant products, including red grapes, mulberries, and peanuts. Resveratrol has been described to exhibit chemopreventive, cardioprotective, antioxidant, anti-inflammatory, and anti-aging activity [1–5]. As a result, resveratrol is used as a nutritional supplement. However, current published evidence is not sufficiently strong to justify administration of resveratrol to humans [6].

There are several hypotheses for the molecular mechanisms that explain the pleiotropic effects of resveratrol. It has been suggested that resveratrol changes the gene expression pattern of the cells.

Abbreviations: bZIP, basic region leucine zipper; MAP kinase, mitogen activated protein kinase; SRE, serum response element; SRF, serum response factor; TCF, ternary complex factors; TPA, 12-*O*-tetradecanoylphorbol-13-acetate

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Initially, resveratrol was proposed to function as a *bona fide* activator of the SIRT1 histone deacetylase and to change the epigenetics of the cells by stimulating SIRT1 activity. Later, the activation function of resveratrol turned out to be an experimental artefact, based on the use of the Fluor de Lys-SIRT1 detection system [7,8], indicating that resveratrol does not directly activate SIRT1. Nevertheless, resveratrol changes the gene expression pattern of the cells by modulating the activity of stimulus-responsive transcription factors including NF-κB, AP-1, Egr-1, p53 and Nrf-2 [9–13]. However, both resveratrol-induced activation and inhibition of transcription have been reported.

Here, we analyzed the impact of resveratrol stimulation on the expression and activity of the zinc finger transcription factor Egr-1, as Egr-1 expression is highly regulated by a variety of extracellular signaling molecules [14–16]. The results of this study show that stimulation of human HEK293 cells with resveratrol upregulates Egr-1 expression and activity involving the protein kinases Raf and extracellular signal regulated protein kinase (ERK). On the transcriptional level, ternary complex factors connect the resveratrol-induced intracellular signaling cascade with enhanced transcription of the Egr-1 gene.

Materials and methods

Cell culture and reagents

HEK293 cells were cultured as described [13]. Stimulation was performed for 24 h in medium containing 0.05% fetal bovine serum with resveratrol (20 μM, Sigma, # R5010), quercetin (20 μM, ENZO, # 385-001-G005), or curcumin (20 μM, Tocris, # 458-37-7). Cells were pre-incubated for 3 h with PD98059 (50 μM, Calbiochem # S513000) before stimulation. All these reagents were dissolved in DMSO. EGF (Promega, Mannheim, Germany, # G5021, dissolved in H_2O as a 100 μg/ml stock solution) was used at a concentration of 10 ng/ml. HEK293-ΔBRaf:ER cells expressing a conditionally active mutant of BRaf were previously described [17]. Stimulation was performed in medium containing 0.05% fetal bovine serum with 4-hydroxytamoxifen (4OHT, Sigma # H7904, dissolved in ethanol) at a concentration of 200 nM for twenty-four hours.

Lentiviral gene transfer

All lentiviral transfer vectors used in this study are based on plasmids pFUW or pFUWG [18]. The transgenes were expressed under the control of the human ubiquitin-C promoter. The lentiviral transfer vectors pFUW-FLAG-REST/Elk-1ΔC, pFUW-FLAG-REST/CREB, pFUW-mycDA-Raf1, pFUW-MKP-1, pFUW-GAL4-Elk-1 and pFUW-GAL4-Sp1 have been described elsewhere [19–24]. The viral particles were produced by triple transfection of HEK293T/17 cells with the gag–pol–rev packaging plasmid, the env plasmid encoding VSV glycoprotein, and the transfer vector [25].

Reporter assays

The lentiviral transfer vectors pFWEBS2[4].luc, pFWEgr1.2.luc, pFWEgr1.1.luc, pFWEgr1SRE.luc, pFW9E3/cCAF.luc and pFWUAS[5]S-p1[2]luc have been described elsewhere [24,26–28]. HEK293 cells were infected with a recombinant lentivirus encoding a promoter/luciferase reporter gene. Cells were incubated for twenty-four hours in DMEM containing 0.05% fetal bovine serum before stimulation. Stimulation with resveratrol (20 μM) or other polyphenols was performed for 24 h. Cell extracts were prepared using reporter lysis buffer [Promega, Mannheim, Germany) and analyzed for luciferase activities as described [29]. Luciferase activity was normalized to the protein concentration.

Western blots

Nuclear extracts or whole cell extracts were prepared as described [30]. 20 μg of nuclear proteins was separated by SDS-PAGE and the blots were incubated with an antibody directed against Egr-1 (Santa Cruz, Heidelberg, Germany, # sc-189), ERK (Santa Cruz, Heidelberg, Germany, # sc-7383), or phospho-ERK (Santa Cruz, Heidelberg, Germany, # sc-153), respectively. An antibody directed against HDAC1 (Santa Cruz, Heidelberg, Germany, # sc-81598) was used as a loading control as described recently [31,32]. To detect FLAG-tagged proteins, we used the M2 monoclonal antibody directed against the FLAG epitope (Sigma-Aldrich, Steinheim, Germany, # F3165), at 1:3000 dilution in TBS. Antibodies against the myc epitope were prepared from CRL-1729 hybridomas, purchased from ATCC. Immunoreactive bands were detected via enhanced chemiluminescence as described [31,32].

Statistics

Statistical analysis were done by using the two-tailed student's *t*-test. Data shown are mean ± SD from two to three independent experiments ($n=2$, $n=3$). Statistical probability is expressed as $^{**}P<0.01$, and $^{***}P<0.001$. Values were considered significant when $P<0.05$.

Results

Resveratrol upregulates Egr-1 expression

Egr-1 is a point of convergence of many intracellular signaling cascades. Thus, we asked whether resveratrol stimulation has an effect on the expression of Egr-1. HEK293 cells were serum-starved for 24 h and then incubated with resveratrol. Egr-1 immunoreactivity was low in the absence of stimulation. In contrast, resveratrol treatment strikingly increased expression of Egr-1 (Fig. 1A). The expression of histone deacetylase-1 (HDAC1) was analyzed as a loading control. An analysis of the time frame revealed that the upregulation of Egr-1 expression started 5–8 h after stimulation, reaching highest levels after 24 h. In contrast, stimulation of the cells with EGF induced a transient expression of Egr-1 with a peak expression occurring 1 h following stimulation (Fig. 1B).

The newly synthesized Egr-1 is biologically active

The ability of Egr-1 to activate transcription depends upon the concentrations of the Egr-1 negative cofactors NAB1 and NAB2. These proteins bind to Egr-1 and block transcriptional activation via Egr-1 [29,33–34]. Thus, elevated Egr-1 protein levels do not automatically indicate an increased transcription of Egr-1 target genes. To measure the biological activity of Egr-1 activity, an Egr-