Resveratrol Inhibits Intestinal Tumorigenesis and Modulates Host-Defense-Related Gene Expression in an Animal Model of Human Familial Adenomatous Polyposis

Yann Schneider, Benoit Duranton, Francine Gossé, René Schleiffer, Nikolaus Seiler, and Francis Raul

Abstract: We studied the effect of oral administration of resveratrol, a natural constituent of grapes, on tumorigenesis in Min mice. Min mice are congenic mice genetically predisposed to develop intestinal tumors as a result of a mutation of the Apc gene. Resveratrol (0.01% in the drinking water containing 0.4% ethanol) was administered for seven weeks to Min mice starting at five weeks of age. The control group was fed the same diet and received water containing 0.4% ethanol. Resveratrol prevented the formation of colon tumors and reduced the formation of small intestinal tumors by 70%. Comparison of the expression of 588 genes in the small intestinal mucosa showed that resveratrol downregulated genes that are directly involved in cell cycle progression or cell proliferation (cyclins D1 and D2, DP-1 transcription factor, and Y-box binding protein). In addition, resveratrol upregulated several genes that are involved in the recruitment and activation of immune cells (cytotoxic T lymphocyte Ag-4, leukemia inhibitory factor receptor, and monocyte chemotactic protein 3) and in the inhibition of the carcinogenic process and tumor expansion (tumor susceptibility protein TSG101, transforming growth factor-β, inhibin-β A subunit, and desmocollin 2). Our data highlight the complexity of the events associated with intestinal tumorigenesis and the multiplicity of the molecular targets of resveratrol. The high potency and efficacy of resveratrol support its use as a chemopreventive agent in the management of intestinal carcinogenesis.

Introduction

Resveratrol (trans-3,4′,5-trihydroxystilbene) is a natural antifungal agent found in grapes and a variety of medicinal plants (1). Because of its high concentration in grape skin, significant amounts of resveratrol are present in wines. Owing to the procedure, <0.1 mg/l of resveratrol is found in white wines. In contrast, red wines contain up to 8 mg/l (2). It has been proposed that this high resveratrol content may explain in part the apparent ability of moderate consumption of red wine to reduce the risk of cardiovascular disease (3). More recently, there has been increasing interest in resveratrol because of reports on its anticarcinogenic effects as assessed by analyzing the major stages (initiation, promotion, and progression) of malignant transformation (4). The molecule has antioxidant and anti-inflammatory properties, and it seems able to inhibit cyclooxygenase and hydroperoxidase activities (4). Recent reports have demonstrated that resveratrol is able to inhibit ribonuclease reductase and DNA synthesis (5) and to arrest cell cycle progression at the S/G2 or G1/S phase transition (6,7).

On the basis of the above-reported findings and the potential relevance of resveratrol as a promising chemopreventive component of human diets, we investigated the effects of orally administered resveratrol on the progression of carcinogenesis in Min (multiple intestinal neoplasia) mice genetically predisposed to develop intestinal tumors. Min is a mutant allele of the murine Apc (adenomatous polyposis coli) locus, encoding a nonsense mutation at codon 850. Min mice develop a variety of tumors and lesions similar to those seen in humans carrying germline mutations in the Apc gene and developing familial adenomatous polyposis, an inherited colon cancer syndrome (8). Apc is the most frequently mutated gene in sporadic colon tumors in humans. In contrast to humans, where the colon is more heavily involved, the small intestine is the preferred site of tumor development in Min mice.

We report data showing that oral administration of resveratrol initiates a dramatic decrease in the number of tumors in the small intestine and completely suppresses tumor formation in the colon of Min mice. By analyzing differential gene expression patterns in the mucosa of the small intestine, we were able to show that resveratrol downregulated a panel of genes directly involved in the progression of tumorigenesis and upregulated several genes controlling ge-
nome stability and cellular differentiation and favoring the activation of antitumoral defense mechanisms.

**Materials and Methods**

**Animals and Diets**

The experiments were conducted according to the National Research Council Guide for the Care and Use of Laboratory Animals with the authorization (no. 00573) of the French Ministry of Agriculture.

C57BL/6j-ApcMin male mice were five weeks old at time of purchase from Jackson Laboratories (Bar Harbor, ME). They were housed under standardized conditions: 22°C, 60% relative humidity, 12:12-hour light-dark cycle, and 20 air changes per hour. The mice were randomly divided into two groups and fed ad libitum a standard pelletted diet (reference no. 105, UAR, Villemoisson, France) that has a crude protein content of 22%, a crude fat content of 5%, and a crude fiber content of 4%.

One group (n = 10) of five-week-old mice received drinking water containing 0.01% resveratrol (Sigma-Aldrich, Saint Quentin Fallavier, France). This amount of resveratrol is ~10 times the amount found in 1 liter of Pinot Noir red wine. Resveratrol was first dissolved in 0.4 ml of absolute ethanol and added to 100 ml of drinking water. The control group (n = 10) received the same volume of drinking water containing 0.4% ethanol.

All mice had free access to the drinking solution between 5 PM and 8 AM. The mice consumed 3–4 ml of the drinking fluid daily. The daily consumption of resveratrol was 0.3–0.4 mg/mouse. After seven weeks, the entire colon and the jejunileum (from the ligament of Treitz to the cecum) were collected under anesthesia and flushed with ice-cold 0.9% NaCl.

**Assessment of the Number of Tumors in the Jejunoileum and Colon**

Seven mice of each group were used for the morphological studies. The jejunileum and colon from each mouse were scored for tumors by examination of 5-cm intestinal sections along the entire jejunileum and colon. Tumors were scored for tumors by examination of 5-cm intestinal sections along the entire jejunileum and colon. Tumors were counted under a dissecting microscope at ×10 magnification after the intestine had been cut open and pinned flat. All tumor counts were performed by a single blinded observer.

**Semiquantitative Monitoring of Gene Expression Patterns With cDNA Expression Arrays**

Of each treatment group, the mucosa of the jejunileal segment of three mice was collected separately by scraping the normal-appearing mucosal surface with a sterile glass slide; tumor tissue was not present in the collected samples. The mucosal samples were weighed in a prechilled sterile tube before addition of a denaturing solution (2.7 M guanidine thiocyanate, 1.3 M ammonium thiocyanate, 0.1 M sodium acetate, pH 4.0). Total RNA was extracted using the Atlas Pure RNA Isolation Kit (Clontech Laboratories, Palo Alto, CA) following the user’s manual. The purified total RNA (2 μg/sample) was used for cDNA synthesis by reverse transcription using the Atlas Mouse cDNA Expression Array procedure and [α-32P]dATP (Clontech Laboratories). The Atlas Mouse cDNA Expression Array includes 588 mouse cDNAs spotted in duplicate on a positively charged nylon membrane. The array contains coding sequence-specific polymerase chain reaction products derived from completely sequenced cDNA clones. All genes (cDNA) present in the commercially available mouse arrays are identified on a website (www.clontech.com). These genes represent several different functional classes, including cell cycle regulators, tumor suppressors, transcription factors, growth factors, chemokines, and cytokines. Plasmid and bacteriophage DNAs are included as negative controls to confirm hybridization specificity, along with several housekeeping cDNAs as positive controls for normalizing mRNA abundance. The 32P-labeled cDNA probes prepared from 2 μg of total RNA isolated from the intestinal mucosa of control and resveratrol-treated mice were hybridized overnight on separate identical Atlas Mouse cDNA Expression Array membranes. After a high-stringency wash, the hybridization patterns were analyzed and quantified using a PhosphorImager (model 445SI, Molecular Dynamics, Sunnyvale, CA) and ImageQuant software. Transcript abundance was normalized using a set of housekeeping genes.

Three control and three resveratrol-treated mice were arbitrarily paired and compared on separate membranes. The changes in gene expression were well reproduced.

The method is semiquantitative. In a given tissue it allows the comparison between two populations of mRNA that reflect changes in gene expression. To exclude technical artifacts, we considered only changes in mRNA abundance exceeding 1.6-fold variations relative to controls. Under these conditions, differences were consistently recorded for 18 of 588 genes in samples obtained from resveratrol-treated and control animals.

**Statistics**

Values are means ± SE. Statistical differences between groups were evaluated by one-way analysis of variance plus comparison of means. Differences were considered significant at p < 0.01.

**Results**

Drinking fluid and diet consumption was comparable in both groups of mice. Initial mean body weight was 25 ± 0.3 g; it reached 27 ± 0.6 g in the control group and 29 ± 0.4 g in the resveratrol-treated group at the end of the seven-week
Table 1. Effect of Resveratrol on Development of Tumors in Small Intestine and Colon of Min Mice<sup>a–c</sup>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Small intestine</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30 ± 4</td>
<td>4 ± 0.5</td>
</tr>
<tr>
<td>(21–45)</td>
<td>(2–6)</td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td>9 ± 1*</td>
<td>0</td>
</tr>
<tr>
<td>(2–11)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>: Resveratrol (0.01%) was administered orally in drinking water.
<sup>b</sup>: Values are means ± SE of 7 animals/group; ranges are in parentheses.
<sup>c</sup>: Statistical significance is as follows: *, p < 0.01.

Effect of Resveratrol on Tumor Formation in the Jejunileum and Colon

The number of adenomas was reduced by 70% in Min mice receiving 0.01% resveratrol in the drinking water for seven weeks (Table 1). Only a few tumors were present in the colon of nontreated Min mice; the colon of mice treated with resveratrol was tumor free.

Changes in Gene Expression in the Small Intestinal Mucosa After Resveratrol Treatment

The administration of resveratrol led to dramatic changes in the expression of 18 genes as measured by the abundance of their mRNAs among 588 genes tested simultaneously using the Atlas Mouse cDNA Expression Array procedure (Figure 1). Resveratrol downregulated the expression of genes controlling cell cycle progression and transcription. As shown in Figure 2, compared with control Min mice, the mice receiving resveratrol exhibited a highly significant decrease in the expression of 5 genes of a panel of ~180 genes involved in the regulation of cell cycle and the expression of transcription factors and DNA binding proteins: RNA polymerase 1 termination factor TTF1 (~3-fold), cyclins D1 and D2 (~2.6- and ~2-fold, respectively), DP-1 cell cycle regulatory transcription factor (~2-fold), and YB-1 DNA binding protein (~2.6-fold).

In contrast, administration of resveratrol initiated the up-regulation of 13 genes of a panel of ~250 genes involved in control of tumor growth, cell differentiation, or control of the immune response. Among these genes, TSG101 tumor susceptibility protein (+1.8-fold), Mas protooncogene (+1.8-fold), MAP kinase (+2-fold), homeobox protein 4.2 (+1.7-fold), monocyte chemotactant protein 3 (+2.6-fold), leukemia inhibitory factor receptor (+2-fold), glutamate receptor NMDA2B (+5.5-fold), cytotoxic T lymphocyte Ag-4 (+5.5-fold), desmocollin 2 (+4-fold), follistatin (+2.3-fold), thrombopoietin (+2-fold), and two genes of the transforming growth factor-β (TGF-β) family, inhibin-β A subunit (+2.3-fold) and TGF-β (+1.7-fold), were most prominently stimulated.

Discussion

Several epidemiological studies demonstrated that dietary factors inhibit the development of many types of cancers through a chemopreventive mechanism. On the basis of in vitro and some in vivo studies (3–7,9), the antimutagenic and chemopreventive properties of resveratrol are now increasingly recognized. It was recently shown that resveratrol exerts antiproliferative effects on human colonic cancer cells (10) and has a protective role in colorectal aberrant crypt foci formation in rats (11). In this report, we have investigated the effects of oral administration of resveratrol to transgenic Min mice genetically predisposed to develop intestinal tumors, an animal model of human familial adenomatous polyposis. When given orally, resveratrol inhibited the carcinogenic process in small intestine and colon of Min mice, despite the constitutive mutation of the tumor-suppressor gene Apc, which is an early and frequent event in the process of intestinal carcinogenesis. This indicates that an initiated carcinogenic process might be suppressed by a chemopreventive agent present in the diet.

Several cellular and molecular targets that may explain the chemopreventive effects of resveratrol have been identified. The cell cycle machinery and the pathways of reactive oxidants are mostly recognized as sites of resveratrol action (5–7,12). By comparing the expression of 588 selected genes in the intestinal mucosa of control Min mice with that in resveratrol-treated Min mice, we were able to show that resveratrol administration led to major changes in the expression of 18 genes, with variations exceeding 60% compared with controls. Resveratrol downregulated the expression of cyclins D1 and D2, which are directly involved in cell cycle progression. They are generally stimulated during malignancy (13) and repressed by anticancerous phytochemicals (14). Resveratrol reduced significantly the expression of transcription factors, including the DP-1 transcription factor, which is strongly involved in the control of cell proliferation (15).

The present results confirm the antiproliferative properties of resveratrol observed in vitro on several cancer cell lines (4–7,10). Resveratrol has also been shown to inhibit DNA synthesis and to arrest the cell division cycle at the S/G2 phase transition in human intestinal cancer cells (10).

In the present study we show that resveratrol repressed the expression of the Y-box binding protein, which binds to inverted CCAAT box sequences that are present in the promoter region of many genes. Interestingly, it was reported recently that Y-box binding protein is overexpressed in human cancer cells resistant to chemotherapy (16). By downregulating the expression of this transcription factor, resveratrol may lower the resistance of cancer cells to anticancer effectors, such as cytokines and chemokines generated through the stimulation of the immune response. In this regard, our results show that resveratrol upregulated the expression in

104 Nutrition and Cancer 2001
Figure 1. Differential gene expression in normal-appearing intestinal mucosa of a control and a resveratrol-treated Min mouse. $^{32}$P-labeled cDNA probes were prepared in parallel from 2 μg of total RNA isolated from intestinal mucosa of control and resveratrol-treated Min mice (3 pairs). Probes were hybridized to separate and identical Atlas Mouse Array membranes, and hybridization patterns were analyzed by phosphorimaging. A representative pair of cDNA patterns is shown from a control (top panel) and a resveratrol-treated mouse (bottom panel). Important differences were consistently recorded for 18 of 588 genes analyzed between samples obtained from resveratrol-treated and control animals. Ratio of intensities of hybridization signals of ubiquitin (Std 1) to glyceraldehyde 3-phosphate dehydrogenase (Std 2) was used for normalization and for comparison of transcript abundance in both membranes. A: oncogenes, tumor suppressors, and cell cycle regulators. 1n, TSG101 tumor susceptibility protein (GenBank access no. U52945); 2j, RNA polymerase 1 termination factor TTF-1 (X83974); 5l, Mas protooncogene (X67735); 6f, cyclin D1 (S78355); 6g, cyclin D2 (M83749). B: stress response, intracellular transducers, and ion channels and transport. 5m, mitogen-activated protein kinase, p38 (U10871). D: transcription factors and DNA-binding proteins. 2g, DP-1 cell cycle regulatory transcription factor (X72310); 4e, homeobox protein 4.2 (J03770); 7j, YB-1 DNA binding protein (X57621). E: receptors, cell surface antigens, and cell adhesion. 1k, monocyte chemoattractant protein 3 (S71251); 1l, leukemia inhibitory factor receptor (D26177); 5j, glutamate receptor (D10651); 6k, CTLA-4 (X05719); 6l, desmocollin 2 (L33779). F: cell-to-cell communication, cytokines, chemokines, cytoskeleton, and protein turnover. 1l, follistatin (Z29532); 2h, inhibin-β A subunit (X69619); 4e, thrombopoietin (L34169); 4f, transforming growth factor-β (M13177). Housekeeping genes: Std1, ubiquitin; Std 2, glyceraldehyde 3-phosphate dehydrogenase; Std3, β-actin; Std 4, ribosomal S19 protein.
the intestinal mucosa of 3 genes of a panel of 22 genes that are directly involved in the recruitment and activation of immune cells, such as T lymphocytes and macrophages. Thus resveratrol stimulated the expression of cytotoxic T lymphocyte Ag-4, which is an important T cell regulatory molecule, playing an important role in the differentiation of T cells in vivo (17), of leukemia inhibitory factor receptor, a molecule implicated in growth arrest and macrophage differentiation (18), and of the monocyte chemotactic protein-3, which has been shown to activate monocytes, dendritic cells, lymphocytes, and natural killer cells (19). These events strongly suggest that the increased recruitment of active immune cells by resveratrol may be an important clue in the chemopreventive effects of this molecule, by decreasing the rate of neoplastic development and by reducing tumorigenicity. This hypothesis is supported by the recent observation that resveratrol seems to be an important cofactor in anti-inflammatory and anticancer nonspecific immune reactions (20). Furthermore, resveratrol has been shown to interfere with arachidonate metabolism, by reducing the levels of prostanoids as a result of inhibition of cyclooxygenase, and prostaglandin H synthase activities (4,21).

The chemopreventive effects of resveratrol may also be related to the inhibition of tumor invasiveness through the activation of desmocollin 2, which is one of the desmosomal components involved in suppression of tumor spreading (22), and also through the upregulation of a tumor susceptibility gene (TSG101), the inactivation of which in mice results in genome instability, cell transformation, and the ability to form metastatic tumors in nude mice (23). Another target for resveratrol seems to be genes of the TGF-β family: TGF-β, the inhibin-β A subunit, and follistatin, which are well-known growth factors, play an important role in the control of the progression of colorectal cancer (24).

Resveratrol, a phytoalexin, has major biological functions in plants and grapes, particularly protection against fungal infections. Our study highlights the complexity and multiplicity of the molecular targets of the chemopreventive actions of resveratrol. When given orally to animals predisposed to intestinal carcinogenesis, it is able to inhibit tumorigenesis and to induce in the mouse intestinal mucosa cells important changes in the expression of ~3% of the 588 screened genes involved in the control of genome stability, cell growth, and differentiation, by favoring the downregulation of genes known to be implicated in the progression of carcinogenesis and by activating genes involved in the host-defense mechanisms against tumorigenesis and tumor spreading.

The use of a powerful tool such as the simultaneous comparison of differential gene expression has allowed us to discover new aspects of the chemopreventive properties of resveratrol. This type of global approach should become more general as a preliminary search of genetic targets of potential chemopreventive agents.

Acknowledgments and Notes

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