

Chemopreventive effects of Resveratrol on Colorectal Cancer – A Review of *In-vitro*, *In-vivo* mechanisms.

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ABSTRACT

Colorectal cancer (CRC) is one of the leading causes of death worldwide. The development of colorectal cancer involves a multi-step process (adenoma-carcinoma sequence), which is characterized by genetic alterations of oncogenes and tumor suppressor genes. Dietary plant phytochemicals have received considerable attention for their potential role in reducing colorectal cancer risk. Resveratrol (*trans*-3, 4, 5-trihydroxystilbene) is mainly found in grapes, red wine, peanuts, and berries and its colorectal cancer chemoprevention potential is attributed to inhibition of cellular events involved in tumor initiation, promotion and progression. The responsible mechanisms include antioxidant, anti-proliferative, anti-inflammatory, proapoptosis, antiangiogenic and antikinase activities. Resveratrol confer inhibitory effect on premalignant and cancerous cells through suppression of cyclooxygenase, ornithine decarboxylase, heat-shock proteins, and activation of caspase and mucins. Moreover, resveratrol also include up regulates or down regulates various transcription factors resulting in inhibition of inflammation, colony formation and cell growth of colorectal cancer cells. The aim of this review is to highlight the chemopreventive activity of resveratrol towards management of colorectal cancer, with particular focus on the cellular and molecular mechanisms by which such effects might be exerted *in vitro* and *in vivo*.

INTRODUCTION

Etiology of colorectal cancer

Colorectal cancer (CRC) is the second most fatal type of cancer worldwide ^[1]. Colon carcinogenesis is a multistep process characterized by the accumulation of genetic alterations involving a variety of oncogenes (*kras*, *braf*, *pik3ca*, *pten*) and tumor suppressor genes (*apc*, *β-catenin*, *tp53*, *bax*, *smada*, *tgfbr2*) ^[2]. In hereditary CRC, inherited genetic mutations occur in critical genes, such as tumor suppressor genes and the genes associated with DNA mismatch repair. However, sporadic CRCs are due to somatic genetic mutations because of exposure to environmental factors such as dietary carcinogens ^[3]. Chronic inflammatory bowel disease is also an etiologic factor in the development of CRC due to the high oxidative stress burden in the inflamed mucosa, which alters important cellular functions ^[4]. Dietary factors are responsible for 70% to 90% of CRC cases, and are considered as one of the major factors accounting for the variability in cancer incidence and mortality in different parts of the world ^[2]. The use of plant phytochemicals for cancer chemoprevention is widely considered to be an alternative strategy for the management of the disease.

Source and metabolism of resveratrol

Resveratrol (3,5,4O-trihydroxy-trans-stilbene), a phytoalexin, is found in moderate to high quantities in various foods including grapes, dry grape skins, red and white wines, grape juices, peanuts and rhubarb dry root. *Trans*-resveratrol is metabolized to dihydroresveratrol, sulphates and glucuronides conjugates ^[4]. The bioactivity of

the metabolites is still unclear but generally dihydroresveratrol has stronger effect than resveratrol in cell growth inhibition [5].

CHEMOPREVENTIVE EFFECTS OF RESVERATROL ON COLORECTAL CANCER: *IN VITRO* AND *IN VIVO* MODELS

Resveratrol possess a spectrum of chemopreventive properties against precancerous or cancer cells. The chemopreventive effect of resveratrol is associated with its ability to block all three stages (initiation, promotion, progression) of carcinogenesis by modulating signal transduction pathways that control cell division and growth; apoptosis, inflammation, angiogenesis, and metastasis [4,5]. The antitumor activity of resveratrol has been demonstrated in human cell lines and animal models of CRC [6] and there is increased interest in identifying biochemical and molecular targets of resveratrol towards colorectal cancer chemoprevention as deliberated below.

Suppression of tumor growth: aberrant crypt foci and mucin-deficient foci

Colorectal cancer develops through a multistep process in which normal crypts are initiated to form foci of aberrant crypts (ACF) and mucin-deficient foci (MDF) that proliferate by crypt fission to form micro-adenoma (MA) [5]. The MA enlarges to give macroscopic adenoma, adenomatous polyps, and finally adenocarcinoma. As a potential anti-cancer agent, resveratrol can inhibit or retard the growth of experimental tumorigenesis in animal models. Several studies have shown oral administration of *trans*-resveratrol in 1, 2 dimethylhydrazine (DMH)-treated rats, resulted in significant reduction of preneoplastic markers of colon carcinogenesis. According to Alfaras *et al.*, [4], a reduction in total ACF (50%) and MDF (45%) was reported after oral administration of *trans*-resveratrol (60 mg/kg daily) for 49 days. Other studies by Tessitore *et al.*, [7] (200 µg /kg *trans*-resveratrol for 100 days) and Sengottuvelan *et al.*, [8] (8 mg /kg *trans*-resveratrol for 30 weeks) also showed reduction in ACF. Furthermore, a 70% reduction in colon tumors was observed after oral administration of 15 mg/kg *trans*-resveratrol for 7 weeks [9]. However, Ziegler *et al.* [10] reported non-significant decrease of adenomatous polyposis upon administration of 4, 20, 90 mg/kg *trans*-resveratrol for 7 weeks using the Apc Min⁺ mice model. Nevertheless, the differences in the outcomes of the *in vivo* models have been attributed to factors such as dosage levels, delivery method, diet components, presence of other dietary components, and tumor origin [2]. The down regulation of inflammation, cell proliferation, and mucosal integrity and up regulation of apoptosis has been suggested as potential mechanisms for the ACF and MDF reductions by resveratrol [5].

Anti-inflammatory implications: suppression effect on cyclooxygenase 2 (COX-2)

The roles of COX-2 and prostanoids in carcinogenesis are supported by the elevated expression of COX-2 and production of prostaglandins E (PGE₂) in colon cancer [2, 6]. Further evidence include the increased susceptibility of COX-2-transgenic mice to chemically induced carcinogenesis, the abrogation of experimental tumorigenesis in COX-2 knock-out animals, and the enhanced skin tumorigenesis after topical administration of a COX-2 product (15-deoxy-D12, 14-prostaglandin J2, 15d-PGJ2) [1, 2, 6]. Prostaglandins such as PGE₂ are mediators of inflammation and are pro-tumorigenic. They are involved in inhibition of apoptosis, induction of angiogenesis, promotion of metastasis and subversion of immune system. Thus COX-2, which is the key enzyme in PGE₂ synthesis, is a potential anti-inflammation target in colon cancer therapy. The chemopreventive effect of resveratrol in Apc Min⁺ mice has been associated with inhibition of cyclooxygenase (COX) enzymes and interference with PGE₂ generation. A study by Sengottuvelan *et al.* [8], showed oral administration of *trans*-resveratrol (8 mg/kg for 30 weeks) inhibited COX-2 in DMH-treated rats. In other studies, resveratrol significantly inhibited the expression of COX-2 in lipopolysaccharide (LPS)-stimulated mouse peritoneal macrophages, and liposaccharide (LPS) / interferon-α (IFN-α)-treated RAW 264.7 macrophages [2, 6]. Resveratrol also down-regulated the expression of COX-2 mRNA transcript in N-nitrosomethylbenzylamine (NMBA)-induced esophageal tumors in F344 rats [11].

Resveratrol can inhibit COX-2 activity either by direct binding or down regulation of transcription factors [6]. In a study aimed at determining the kinetics of interaction between resveratrol and COX-2, Zykova *et al.* [12], reported that resveratrol (50 µM) was able to bind with COX-2, which resulted in inhibition of PGE₂ production, colony formation and cell growth of HT-29 cancer cells. In addition, resveratrol (60 µM) suppressed cell growth in COX-2^{+/+}, but were ineffective in COX-2^{-/-} murine embryonic fibroblasts (MEF) cells, suggesting that COX-2 might be the primary target of resveratrol. In a study by Jang *et al.*, [13], resveratrol 85 µM suppressed hydroperoxidase activity of COX-2, while Subbaramaiah *et al.* [14] observed suppressed COX-2 expression after application of resveratrol (30 µM) in 184B5/HER cells. The binding mode between COX-2 and hydroxylated analogues of resveratrol is attributed to the hydrogen bonding with amino acid residues Arg120, Ser530, and tyr385 [15].

Modulation of cell proliferation: suppression effect on ornithine decarboxylase

The inhibition of abnormal cell proliferation via modulation of cell cycle progression is one of the important strategies for CRC chemoprevention and chemotherapy. Ornithine decarboxylase (ODC) is a rate-limiting enzyme involved in the polyamine biosynthesis and cell proliferation, thus considered a biochemical marker of tumor

promotion^[16]. Elevated ODC expression and cellular polyamine levels are experienced in colon cancer cells due to the higher need for polyamines to sustain growth of neoplastic tissues (DNA, RNA synthesis)^[17]. Oral administration of *trans*-resveratrol (8 mg/kg for 30 weeks) inhibited ODC activity in DMH-treated rats^[8] while resveratrol suppressed ODC activity via *de novo* synthesis of ceramides in human colon cancer (Caco-2) cells^[18]. Various cyclins, cyclin-dependent kinases (Cdk), Cdk inhibitors, and check point kinases (Chk 1, Chk 2) are involved in the signalling of homeostatic maintenance of cell growth and differentiation^[19]. Therefore, the suppression of abnormal cell proliferation by down-regulating cyclin-Cdks or upregulating Cdk inhibitors are potential targets in chemoprevention and chemotherapy of CRC by dietary phytochemicals^[16]. Resveratrol can inhibit inappropriate proliferation and growth of various cancer cells in culture, tissues from animals exposed to carcinogens by modulating the cyclins, Cdk, Cdk inhibitors, and check point kinases that are involved in regulating cell cycle progression^[2].

Modulation of cell apoptosis: induction of caspase and inhibition of heat-shock proteins

The selective induction of apoptosis in premalignant or cancerous cells cancer cells is regarded as an important strategy for CRC prevention and therapy. Resveratrol can induce apoptosis in various cancerous or transformed cells by activating both extrinsic and intrinsic pathways of cell death machinery^[20]. Resveratrol induces apoptosis by activating pro-apoptotic signaling molecules as well as inhibiting anti-apoptotic molecules of the intracellular signal transduction pathways^[2]. Oral administration of *trans*-resveratrol (8 mg/kg for 30 weeks) activated expression of caspase-3, a precursor of pro-apoptotic cascade, in DMH-treated rats^[8]. In other studies, resveratrol induced apoptosis in chemically induced mouse skin papillomas via induction of p53, release of cytochrome c, activation of Bax, inhibition of Bcl-2, and processing of caspases (caspase 9, caspase 3) and cleavage of poly-(ADP) ribosylpolymerase (PARP)^[5, 10, 21]. Resveratrol can also activate caspase-2 and caspase-8, resulting in the processing of down-stream caspases and cell death in a death receptor or mitochondria-independent manner^[20]. According to Dong^[5], pretreatment of human prostate cancer (PC-3, DU-145) cells with resveratrol resulted in Trail-, Fas- or TNF- α -mediated cells death by multiple mechanisms involving down-regulation of inhibitor of apoptotic protein (IAPs), suppression of Akt phosphorylation, and subsequent activation of caspase. Over expression of heat-shock proteins (HSP) results in blocking of the caspase processing during apoptosis. In a study by Sengottuvelan *et al.*,^[8] oral administration of *trans*-resveratrol (8 mg/kg for 30 weeks) inhibited HSP70 and HSP27 expression in DMH-treated rats.

Modulation of mucosal integrity: implication of mucins (MUC-2, MUC-1)

Mucins such as MUC-2 are importance in the maintenance of mucosal integrity. MUC-2 deficiency has been associated with inflammation of the colon, leading to onset of colitis^[22]. Oral administration of resveratrol (8 mg/kg for 30 weeks) activated the expression of MUC-2 and inhibited expression of MUC-1 in DMH-treated rats, thus resveratrol maintained the normal integrity of the colon^[8]. The effect of resveratrol is attributed to the modulation of the enzymes that initiate o -glycosylation of mucin^[2].

Regulation of transcription factors by resveratrol

The multistage carcinogenesis involves disruption of the normal intracellular signaling network. An array of receptor proteins, upstream kinases, DNA-interacting proteins, and transcriptionally regulated gene products function abnormally during the course of carcinogenesis, thereby favoring premalignant and malignant transformation of damaged cells^[23]. Evidence from cultured cells and experimental animals indicate that resveratrol can modulate abnormal turning on or switching off various upstream kinases and transcription factors^[15]. Resveratrol is capable of modulating mitogen-activated protein kinase (MAPK) transduction pathways by up-regulating c-Jun, NH₂-terminal kinases 1 and 2, and p38 but down-regulates extracellular regulated kinase (ERK-1, ERK-2), Src tyrosine kinase, focal adhesion kinase, protein kinase C isoform α and γ ; protein kinase B, and kappa B kinase inhibitor^[1, 2, 6]. It can also down-regulate the transcriptional factors such as early growth response protein 1, activating protein 1 transcription factor, c-myc, c-Fos, p65, nuclear factor κ B (NF- κ B) subunit, and inhibit epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) activation^[1, 2, 6]. Overall, there have been very limited clinical studies on the inhibitory effect of resveratrol on colorectal cancer^[24, 25]. However, the focus of these studies has been on the bioavailability of resveratrol. Thus, further human studies would provide additional knowledge on the potential role of resveratrol in management of colorectal cancer. In particular, the design of such trials should focus on identifying possible interactions with other dietary factors, and development of bioavailable analogues of the compound.

CONCLUSION

Resveratrol are promising chemopreventive agents for CRC management because of their inhibitory effect on premalignant and cancerous cells. Resveratrol targets multiple molecular and biochemical pathways implicated in tumor development, including suppression of cyclooxygenase, ornithine decarboxylase, heat-shock proteins, and

activation of caspases and mucins. Moreover, resveratrol may also up regulate or down regulate various transcription factors resulting in inhibition of COX-2 mediated PGE₂ production, colony formation and cell growth of colorectal cancer cells. The available *in vitro* and *in vivo* data suggest that resveratrol might be a promising phytochemical to be applied for the colorectal cancer chemoprevention.

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