Minireview

Targeting extracellular matrix remodeling in disease: Could resveratrol be a potential candidate?

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Abstract

Disturbances of extracellular matrix homeostasis are associated with a number of pathological conditions. The ability of extracellular matrix to provide contextual information and hence control the individual or collective cellular behavior is increasingly being recognized. Hence, newer therapeutic approaches targeting extracellular matrix remodeling are widely investigated. We reviewed the current literature showing the effects of resveratrol on various aspects of extracellular matrix remodeling. This review presents a summary of the effects of resveratrol on extracellular matrix deposition and breakdown. Mechanisms of action of resveratrol in extracellular matrix deposition involving growth factors and their signaling pathways are discussed. Involvement of phosphoinositol-3-kinase/Akt and mitogen-activated protein kinase pathways and role of transcription factors and sirtuins on the effects of resveratrol on extracellular matrix homeostasis are summarized. It is evident from the literature presented in this review that resveratrol has significant effects on both the synthesis and breakdown of extracellular matrix. The major molecular targets of the action of resveratrol are growth factors and their signaling pathways, phosphoinositol-3-kinase/Akt and mitogen-activated protein kinase pathways, transcription factors, and SIRT-1. The effects of resveratrol on extracellular matrix and the molecular targets appear to be related to experimental models, experimental environment as well as the doses.

Keywords: Resveratrol, extracellular matrix, matrix metalloproteinases, TGF-β

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Introduction

Extracellular matrix (ECM) is the collection of several proteins and sugars that form a matrix around the cells in all solid organs. The insoluble scaffold formed by ECM provides not only the structural support but also creates an essential milieu to support the fundamental cellular functions.[1] The ability of ECM to provide contextual information and hence control the individual or collective cellular behavior is increasingly being recognized. Disturbances of ECM remodeling are known to be associated with a number of pathological conditions such as cancer and fibrotic diseases. Accordingly, newer therapeutic approaches targeting ECM remodeling are increasingly being investigated. Resveratrol, a polyphenol from grapes and berries, has undergone extensive investigations for its benefits in a wide range of diseases. This review presents a summary of literature to understand the effects of resveratrol on various aspects of ECM remodeling and to assess if resveratrol could be a potential candidate for the treatment of diseases involving disruption of ECM homeostasis. The literature search for this review was made using Pubmed search engine. A total of 90 papers were included and the abstracts as well as full text papers of all included studies were appraised.

Extracellular matrix: composition, function, and remodeling

ECM consists of a complex network of elastic fibers, collagens, and non-collagenous glycoproteins. Among all, collagens are most abundant; however, individual ECM components are differentially expressed in different tissues. For example, major arteries, cartilages, tendons and ligaments consist of ECM rich in chondroitin sulfate which gives them the required tensile strength. ECM also consists of basement membranes (BM) made up of laminins, entactin, collagen IV, and heparan sulfate that provide an anchoring surface for epithelial cells. The composition and concentration of ECM components determine its biochemical properties and is tissue specific.[2] BM in ECM regulate the apicobasal orientation of cells. Alterations in the composition of BM lead to loss of cell polarity, which in turn can promote cell proliferation and tumorigenesis.[3] A precise spatial organization of molecular network confers such

biophysical properties to ECM that allow it to integrate complex signals and provide an ''outside-in'' cellular signaling to enable cells to detect biomechanical and biochemical changes in tissue and respond accordingly.[1,4] ECM signaling regulates cell behavior by modulating cell proliferation, cytoskeletal organization, cellular differentiation, and receptor signaling.[5,6] ECM also acts as a local depot and sequesters several cytokines and growth factors.[7] ECM remodeling is a continuous dynamic process that involves a precise balance of the rate of matrix synthesis, secretion and modification with the rate of its degradation. Enzymes such as hyaluronidases, matriptases, cathepsins, heparanase, metzincins, and serine and threonine proteases are involved in ECM degradation.[8] Metzincins superfamily includes ADAMs (disintegrin and metalloproteinases), ADAMTSs (ADAMs with thrombospondin motifs), matrix metalloproteases (MMPs), and the tissue inhibitors of MMPs (TIMPs).[9]

Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene) is a phytoalexin with a stilbenoid core and is present abundantly in grapes, berries, and peanuts. It exists as two isomers; cis and trans, and between the two, trans-resveratrol is biologically more active. Clinical trials have demonstrated its safety and efficacy in several diseases including hypertension, cardiovascular diseases, colorectal cancer, and Alzheimer disease.[10–13] An extensive review on the properties of resveratrol in animals and human has recently been published.[14] The potential therapeutic benefits of resveratrol in many of these pathological conditions may at least partially be attributed to its effects on ECM remodeling.

Resveratrol and ECM deposition

Shen et al. showed that resveratrol enhances ECM production by degenerative nucleus pulposus cells (DNPCs) and Yaman et al. showed that it increases the hydroxyproline levels, collagen deposition, and neovascularization in rats after laparotomy.[15] Resveratrol was shown to prevent loss of matrix proteoglycan content in cartilage of rabbits with experimental osteoarthritis.[16] Studies, however, have also shown that it inhibits ECM synthesis.[17] In one of the studies, it prevented the advanced glycation end product (AGE)-induced prolyl hydroxylase increase, a marker for collagen synthesis, in vascular smooth muscle cells from stroke-prone spontaneously hypertensive rats in a dose-dependent manner.[18] In fructose-induced rat model of metabolic syndrome, cardiac fibrosis (collagen I accumulation) is associated with expression of osteopontin, a factor induced by reactive oxygen species (ROS) and a collagen I expression inducer. Administration of resveratrol 2.1 mg/kg to these rats prevented expression of both the collagen I and osteopontin.[19] In streptozotocin-induced diabetic rats, resveratrol alleviates vascular wall thickening, collagen deposition/cross-linking, and vascular permeability.[20] Resveratrol (2.5 mg/kg) was also shown to alleviate cardiac fibrosis and functional abnormalities in spontaneously hypertensive rats.[21] Similar antifibrotic effects of resveratrol have been observed in liver,[22,23] GIT,[24–27] urinary tract,[28] lungs,[29,30] pancreatic stellate cells (PSCs)[31] and skin.[32]

Resveratrol and ECM degradation

Among several ECM degrading enzymes, MMPs, a zinc-dependent endopeptidase family, have been investigated most widely. MMPs are at least of 26 types and are classified on the basis of their specificity for different substrates. In recent years, MMPs have been shown to play an important role particularly in cancer metastasis. Additionally, conditions such as chronic inflammation, arthritis, and wrinkle formation are also characterized by excessive ECM breakdown. Hence, reduction in MMPs seems an important therapeutic option in these pathological conditions. On the other hand, increased MMPs would be beneficial in pathological conditions with increased fibrotic changes. While resveratrol largely inhibits ECM deposition, it has variable effects on MMPs. In fact, collective results from several studies indicate dual effects of resveratrol on MMP secretion. Some studies have shown that it reduces MMPs while others have shown that it induces MMPs both at protein and mRNA level. The effects appear rather dose- and context-dependent.

Studies, particularly those using cancer cell lines, have shown that resveratrol inhibits MMP secretion. Human cultured glioblastoma cells showed a dose-related decrease of MMP-2 mRNA and protein and lower Secreted Protein Acidic and Rich in Cysteine (SPARC) gene and protein expression 72 h after resveratrol (1 and 50 μM) treatment.[33] SPARC is a glycoprotein that has counter-adhesive properties.[34] It also takes part in proteolytic pathways by increasing the expression of collagenase and MMP-9, and activating MMP-2.[35] Similar observations have been made in breast cancer cells,[36] glioblastoma-initiating cells,[37] human fibrosarcoma cells[38] and human lung adenocarcinoma cells.[39] Treatment of human brain microvascular endothelial cells (HBMEC) and HepG2 cells with resveratrol after incubation with phorbol ester PMA, a known cancer promoting agent, also resulted in dose-dependent decrease in MMP-9 activity and increase in TIMP-1 protein.[40,41] In contrast, resveratrol caused a dose-dependent increased activation and expression of MMP-9 in HT1080 human fibrosarcoma cells.[42]

Besides tumorigenesis and metastatic progression of cancer, MMPs also play a role in ECM remodeling in response to inflammation. Treatment of U937 cell line, a human promonocytic cell line, with PMA (10 pmol/L – 0.1 μmol/L) induces cell differentiation and increased MMP-9 production that mimics inflammatory conditions. However, co-treatment with resveratrol causes a dose-dependent decrease in PMA-induced MMP-9 production.[43] Release and activation of MMP-2 and −9 from activated endothelial cells in response to tumor necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1 is also inhibited by resveratrol (25 μM).[44] Macrophages pretreated with resveratrol (30 μM) and stimulated with pro-inflammatory 7-oxo-cholesterol also showed significant prevention of the upregulation of MMP-2 and MMP-9.[45]

The neurological damage caused by ischemia-reperfusion injury is also known to involve increased MMP activity. Resveratrol inhibits MMP-9 and −3 expression induced by middle cerebral artery occlusion in mice or by transient oxygen-glucose deprivation in primary cortical neurons.[46,47] Recently, studies have shown that resveratrol provides neuroprotection against ischemia by restoring the balance of MMP-9/TIMP-1 expressions and activities, hence maintaining the integrity of blood-brain barrier.[48] However, in the delayed phase of focal neurological injury, resveratrol seems to cause upregulation of MMP-2 and VEGF, both of which are important in remodeling the ECM components and new vessel formation.[49] Suppression of MMP-2 and MMP-9 was also demonstrated in rats with ethinylestradiol-induced intrahepatic cholestasis of pregnancy[50] and in the retina of rats with high intraocular pressure-induced retinal ischemia.[51]

In contrast to its effects on MMPs in tumour cells or in response to inflammation and ischemia, other studies have shown that resveratrol increases MMP secretion.[52] In one of our recent studies, topical application of resveratrol in rats pretreated with dexamethasone resulted in increased MMP-2 levels in the aqueous humor of rat eyes. Steroid instillation is known to increase the ECM deposition in the trabecular meshwork resulting in reduced aqueous humor drainage and consequently elevated intraocular pressure (IOP).[53] Increased MMP-2 secretion in response to treatment with resveratrol was associated with reduced ECM deposition and reduced IOP.[54] Moreover, in another study, TIMP-1 mRNA expression was found to be reduced in mice with bile duct ligation injury after treatment with resveratrol (4 mg/kg/day) for seven days.[27]

Mechanisms of inhibition of ECM deposition by resveratrol

Resveratrol inhibits growth factors

Resveratrol downregulates growth factors, and in one of the studies, connective tissue growth factor was shown to be downregulated by an oral dose of 17 mg/kg/day in rats 12 weeks after experimentally induced myocardial infarction.[55] The effects of resveratrol on transforming growth factor (TGF)-β have more widely been investigated. In epithelial malignant human tumors and experimental tumors such as squamous cell carcinoma (SCC), TGF-β is overexpressed. Its overexpression promotes epithelial-mesenchymal transition (EMT), progression of tumor and its metastasis.[56] Human SCCs and p53+/−/SKH-1 mice with UVB-induced SCCs also overexpress TGF-β1 and TGF-β2. Treatment with resveratrol, 100 mg/kg, was shown to substantially decrease TGF-β2 expression in a time- and concentration-dependent manner, whereas the effect on TGF-β1 expression was substantially less.[57] Using several cell lines such as A549 (lung cancer cell line), ZR75-1 (breast cancer cell line), HaCaT (keratinocyte cell line), HeLa (uterus cancer cell line) and PANC-1 (pancreatic cancer cell line), Suenaga et al. studied the effects of resveratrol on TGF-β2 promoter-luciferase reporter construct that incorporated human TGF-β2 gene promoter regions.[58] In contrast to the results of the study by Kim et al.,[57] A549 human lung epithelial cells showed a significant increase in the reporter activity in response to treatment with 3 and 10 μM of resveratrol with an increase in the amounts of total TGF-β2. However, TGF-β1 and -β3 proteins did not show any change. Additionally, it was observed that tamoxifen, an estrogen receptor antagonist, abolished this effect of resveratrol, hence indicating that in A549 cells, resveratrol induced the formation of TGF-β2 protein through estrogen receptors. This study also demonstrated that resveratrol initially up-regulates TGF-β2 expression and then the endogenously produced TGF-β2 acts on A549 cells in an autocrine manner.[58] Lu and Serrero have also reported earlier that MCF-7, an estrogen receptor-positive human breast cancer cell line, shows increased expression of TGF-β2 mRNA after treatment with >5 μM resveratrol.[59] The differential effects of resveratrol on TGF-β2 could perhaps be attributed to the differences in the concentration of resveratrol and also the type of cells used.

A number of other studies have shown that resveratrol also significantly affects TGF-β1. In rats with unilateral ureteral obstruction, resveratrol was shown to reduce TGF-β1 expression. In the same study, TGF-β1-induced EMT and ECM synthesis by cultured renal tubular epithelial cells (NRK-52 E), was found to be abolished after treatment with resveratrol.[28] TGF-β1-induced EMT and expression of vimentin and fibronectin was suppressed by resveratrol (20 μM) in A549 lung cancer cells *in vitro*.[60] Similar observations were made on animals models of hepatic fibrosis,[22,23] enterocolitis,[25] allergic airways disease[30] and in MCF-7/TR cells.[61]

TGF-β is also a potent inducer of the differentiation of fibroblasts to myofibroblasts, which are an important source of ECM proteins.[62] Hence, inhibition of fibroblast differentiation is considered a useful strategy to combat fibrosis. In one of the studies, pretreatment with resveratrol prevented TGF-β-induced differentiation of cardiac fibroblasts into myofibloblasts. Besides TGF-β, angiotensin II also stimulates fibroblast differentiation via angiotensin II type 1 (AT1) receptors. The above study also demonstrated inhibitory effect of resveratrol on angiotensin II-induced differentiation of cardiac fibroblasts.[63]

Resveratrol-induced suppression of growth factors involves Smad-dependent and Smad-independent pathways

TGF-β-induced changes in cellular functions are mediated through Smad-dependent as well as Smad independent intracellular pathways.[64] Resveratrol causes dose-dependent decrease in the levels of phospho-Smad2/3 (signal transducers that are downstream targets of TGF-β receptor-I) in mice with UVB-induced SCC and completely abolishes Smad phosphorylation at 100 μM.[57] Inhibition of Smad phosphorylation by resveratrol was also observed in A549 human lung epithelial cell and MCF-7/TR cell line.[58,59] Besides affecting Smad-dependent pathways, resveratrol also affects Smad-independent pathways as indicated by the modulation of the activity of the components of mitogen activated protein kinase (MAPK)

pathways. Vergara et al. demonstrated that resveratrol inhibits epidermal growth factor (EGF)–induced EMT by suppressing extracellular regulated kinase1/2 (ERK1/2) pathway in MCF-7 cells.[65] In a recent study, resveratrol significantly attenuated phosphorylation of p44/p42 MAPK and SAPK/JNK in osteoblast-like MC3T3-E1 cells stimulated by TGF-β; however, the phosphorylation of Smad2 and p38 MAPK remained largely unaffected.[66]

Collagen I synthesis in response to IGF-1 is also known to be mediated through MAPK pathways. Not only the IGF-1-induced but also the basal expression of collagen I gene and protein was inhibited by resveratrol in CCD-^{18}Co cells and intestinal fibroblasts. This inhibitory effect of resveratrol involved IGF-1/IGF-1receptor (R)/ERK1/2 pathway.[26] It was observed that IGF-1-stimulated phosphorylation of IGF-1 R and ERK1/2 was remarkably inhibited after treatment with resveratrol for 30 min without affecting the expression of total IGF-1 R and ERK1/2. This suggests that resveratrol reduces IGF-1 R activity and intracellular ERK signaling cascade. In this study, it was also observed that treatment of CCD-^{18}Co cells with IGF-1 after pretreatment with resveratrol for 30 min effectively downregulates phosphorylation of IGF-1 R compared to treatment with IGF-1 alone.[26] The above data, collectively, suggest that the repression of collagen I synthesis by resveratrol can be attributed to inhibition of IGF-1 R/ERK1/2 signaling.

Mitogenic effect of angiotensin II on cardiac fibroblasts and vascular smooth muscle cells (VSMC) also involves ERK activation; however, transactivation of EGF receptor (EGFR) is an essential intervening step. Resveratrol was shown to inhibit ERK phosphorylation via AT1 as well as EGFR in cardiac fibroblasts and VSMC. Since effects of AT1 stimulation are mediated through multiple signaling pathways, it is likely that resveratrol also inhibits pathways other than MAPK. In line with this assumption, the effect resveratrol on VSMC proliferation was also shown to involve inhibition of phosphatidylinositide 3-kinase (PI3-kinase)/Akt pathway.[63]

Similarly, Akt- cAMP response-binding protein (CREB) signaling is involved in resveratrol-mediated TGF-β2 downregulation.[57] A stable interaction with the co-activators p300 and the CREB-binding protein (CBP) is induced by phosphorylation of CREB at serine 133 which in turn leads to transactivation of target genes.[67] Accordingly, in A431 cells, resveratrol caused dose-dependent decrease in the levels of both phospho-Akt (S473) and phospho-CREB but did not affect total Akt and CREB. Since, inhibition of CREB phosphorylation by resveratrol was only partially antagonized by Akt overexpression, it was concluded that Akt-dependent as well as -independent mechanisms mediate inhibition of CREB phosphorylation by resveratrol.[57]

Resveratrol alters the expression of SIRT-1

Sirtuins (SIRT1-7) are a family of protein-modifying enzymes that belong to class III histone deacetylases (HDACs) and play a key role in regulating gene transcription. SIRT1 seems to be involved in a wide range of diseases. It deacetylates lysine groups of proteins and transcription factors.[68] Deacetylation of RelA/p65 subunit of NF-kappaB at lysine 310 inhibits transcription.[69] Li et al. verified if repression of collagen I by resveratrol involves SIRT1. Fibroblasts transfected with either WT SIRT1 or deacetylase-inactive mutant SIRT1 expression constructs were treated with IGF-1. Collagen I protein expression in response to IGF-1 was markedly decreased in cells with WT but not in those with inactive SIRT1. Furthermore, in CCD-^{18}Co cells, resveratrol-induced reduction of collagen I expression was abolished following SIRT1depletion by siRNA (1 and 3). These results indicate involvement of SIRT1 in the repression of collagen I expression by resveratrol.[26] Resveratrol was also shown to induce SIRT1-dependent autophagy in primary human fibroblasts.[29] Treatment of these cells with resveratrol was found to increase the levels of autophagy markers LC3BII and Atg5. In lung epithelial cells and fibroblasts, increased expression of LC3BII is associated with SIRT1 overexpression. Importantly, resveratrol fails to reduce ECM deposition after SIRT1 silencing. Hence, suggesting that resveratrol decreases ECM deposition in a SIRT1-dependent manner.

Contrary to the results of the studies showing SIRT-1-dependent suppression of ECM deposition by resveratrol, Shen et al. observed the opposite. Treatment of primary alginate-cultured DNPCs with resveratrol 0, 12.5, 25, 50, 100, and 200 μmol/L for 12, 24, and 48 h caused upregulation of the SIRT1 mRNA and protein expression, and this was accompanied with increased ECM expression. They also observed significantly reduced expressions of Colla2α1 and aggrecan after silencing SIRT1 expression by siRNA.[17] Hence, it was concluded that resveratrol stimulates synthesis of ECM by DNPCs in a dose-dependent and SIRT1-dependent manner.

Therefore, it is likely that SIRT-1-dependent effects of resveratrol on ECM deposition are affected not only by dose and the type of cells but also by the type of stimuli modulating ECM deposition.

Mechanisms of action of resveratrol on ECM degrading enzymes

Resveratrol acts on adenosine receptors

Several studies have demonstrated that resveratrol activates adenosine receptors.[70–72] In one of our previous studies, topical application of resveratrol to rat eyes after pretreatment with dexamethasone resulted in reduced IOP and the reduction in IOP was attributed to stimulation of adenosine A1 receptors. Using computational studies, we also observed that trans-resveratrol has highest affinity for A2B and A1, followed by A2A and A3 adenosine receptor subtypes.[73] Adenosine receptor stimulation has been shown to cause increased MMP secretion,[74] and accordingly, resveratrol-induced adenosine receptor stimulation was shown to cause increased MMP-2 secretion.[73]

Resveratrol affects activation of transcription factors

Resveratrol-induced suppression of MMPs gene and protein expression has been shown to involve NF-κB signaling. Resveratrol-induced MMP-3 inhibition in primary cortical neurons exposed to transient oxygen-glucose deprivation

was shown to involve inhibition of NF-κB expression.[51] Annabi et al. showed that treatment of HBMEC with 1 μM phorbol ester PMA led to phosphorylation of inhibitory κB (IκB) that peaked at 15 min. However, IκB phosphorylation was diminished when PMA treatment was done after pre-incubation with 30 μM resveratrol. Migration of NF-κB to nucleus is preceded by its release after phosphorylation of IκB.[40] Pretreatment of macrophages with resveratrol also prevented the upregulation of active NF-κB p50 and p65 in response to proinflammatory 7-oxo-cholesterol in macrophages.[45] Inhibition of NF-κB by resveratrol was also demonstrated by Jiao et al. using glioblastoma-initiating cells. In this study, treatment with resveratrol 10 and 20 μM for 48 h inhibited the nuclear translocation of NF-κB p65, and this effect was confirmed by immunofluorescence assay. Resveratrol also efficiently inhibited the phosphorylation of IKKα/β and IkBα.[37] These results indicated that resveratrol inhibits NF-κB activation, which is the upstream activator for MMP-2 expression. One of the studies using human lung adenocarcinoma cells has shown that resveratrol-induced inhibition of NF-κB activation involves heme oxygenase 1. Inhibition or silencing of heme oxygenase-1 caused downregulation of MMP-2 and MMP-9 due to inhibition of the NF-κB-dependent signaling pathway.[39] In other studies as well, resveratrol inhibited the nuclear translocation of activating protein (AP)-1 in U937 cells[43] and NF-κB in primary cortical neurons exposed to transient oxygen-glucose deprivation.[47] Pretreatment of chondrocytes with resveratrol (25–100 μM) for 24 h followed by stimulation with AGEs (100 μM) for another 24 h was also shown to produce dose-dependent inhibition of DNA-binding activity of NF-κB induced by AGEs. Degradation of IκBα was observed 2 h after treatment as it precedes the NF-κB activation. Phosphorylation of IκBα by IκB kinases (IKK) is required for degradation of IκBα. It was observed that AGEs effectively induced IKKα/β phosphorylation without affecting total IKK. In the presence of resveratrol, the levels of AGEs-induced phosphorylated IKKα/β decreased. Hence, it can be concluded that resveratrol inhibits NF-κB by inhibiting IKKα/β→IκBα→NF-κB →DNA-binding activity. Along with the inhibition of NF-κB activation, this study also demonstrated significant inhibition of AGE-stimulated transcriptional activity of AP-1 after treatment of chondrocytes with resveratrol. Since NF-κB and AP-1 signaling pathways regulate MMP-13, the level MMP-13 was also found to be significantly reduced in this study.[75] In agreement with the results of the *in vitro* studies, resveratrol completely attenuated NF-κB activity in a rat model of colonic anastomotic healing[76] and normalized the expression of NF-κB/p65 in diabetic rats.[77] In contrast to the results of above mentioned studies, Yar et al. observed a 1.2 fold increase in NF-κB expression in the heart tissue of resveratrol-treated (10 mg/kg/day; intraperitoneal for four weeks) streptozotocin-induced diabetic rats compared to untreated rats; however, the difference was not significant.[78] As it was an *in vivo* study, the dose of 10 mg/kg/day may not have provided the high enough concentration of unmetabolized resveratrol as used for *in vitro* studies leading to insignificant increase in the expression of NF-κB.

Aberrant activation of STAT3 (signal transducers and activators of transcription 3), a cytoplasmic transcription factor, has been demonstrated in several types of cancers. It contributes to upregulation of MMP-9 and cancer progression.[79,80] RANTES-induced STAT3 activation in MDA-MB-231 breast cancer cells was inhibited by LYR71. LYR71 caused deacetylation of H3 and H4 histones, which inhibited STAT3 and p300 binding to MMP-9 promoter region and resulted in reduced expression and activity of MMP-9 and suppressed tumor progression.[36]

The effects of resveratrol on NF-κB expression are of dual nature. NF-κB expression was downregulated by 10^{-7} mol/L concentration of resveratrol in human embryonic kidney cells, but was stimulated at 10^{-4} mol/L.[81] It remains unclear if NF-κB activation is involved in resveratrol-induced increase in MMP secretion. Several intracellular signaling pathways may be involved in resveratrol-induced modulation of the activity of transcription factors (Figure 1). Some of them, particularly those involved in NF-κB activity, have been investigated in various studies and are summarized below.

PI-3 K/Akt signaling pathway. Jiao et al. while demonstrating that treatment of glioblastoma-initiating cells with resveratrol 10 and 20 μM for 48 h inhibits the nuclear translocation of NF-κB p65 and phosphorylation of IKKα/β and IkBα, also observed that resveratrol significantly reduced the phosphorylation of Akt and mTOR without affecting their total level.[37] The IGF-1 has also been shown to increase MMP-2 secretion through activation of PI-3 K/Akt signaling pathway. This IGF1-stimulated increase in MMP-2 secretion was shown to be reduced by resveratrol due to inhibition of PI-3 K/Akt signaling pathway in human breast cancer cells.[82] Contrary to the above finding, Gweon and Kim showed that treatment of HT1080 human fibrosarcoma cells with resveratrol (50 μM) for 24 h causes increased MMP-9 secretion due to inhibition of Akt phosphorylation.[42]

Mitogen activated protein (MAP) kinase pathway. The MAP kinases are involved in the regulation of MMPs and are modulated by transcription factors. Liu et al. demonstrated that resveratrol (25–100 μM), along with AP-1, inhibits AGE-induced activation of both ERK and JNK but not p38 in porcine chondrocytes. The JNK activity was suppressed in a dose-dependent manner; however, resveratrol did not affect the total amount of JNK. The results of this study, therefore, indicate suppression of AGE-activated JNK/ERK-AP-1 pathway as the mechanism of MMP-13 inhibition by resveratrol in porcine chondrocytes.[75] Similarly, in another study that used human oral cancer cell line, SCC-9, TPA-induced phosphorylation of ERK1/2, and JNK were inhibited by resveratrol (25–100 μM), but not that of p38. This study also demonstrated that inhibition of ERK1/2 and JNK by specific inhibitor inhibits MMP-9 expression, hence suggesting that resveratrol inhibits MMP activity through MAPK pathways.[83] Contrary to this, Gweon and Kim showed that treatment of HT1080 human fibrosarcoma cells with resveratrol 50 μM results in inhibition of p38 phosphorylation resulting in increased

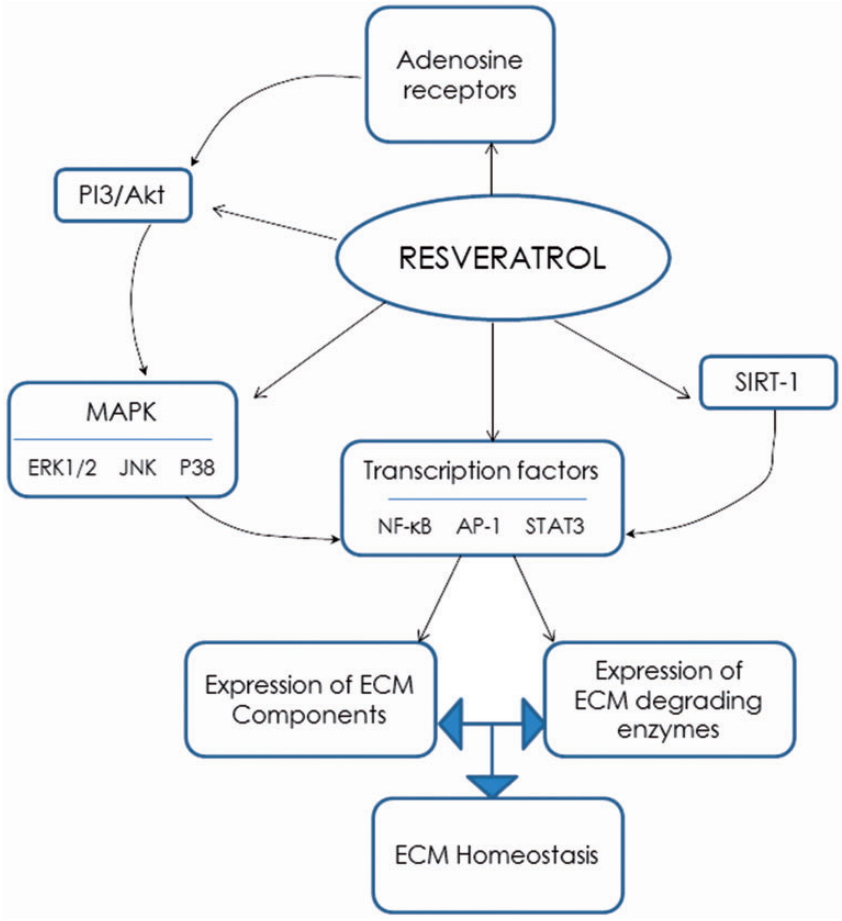

Figure 1 The effects of resveratrol on ECM homeostasis and its molecular targets. PI3: Phosphoinositol-3-kinase; Akt: Protein kinase B; JNK: c-Jun NH(2)-terminal kinase; ERK1/2: Extracellular regulated kinase ½; JNK: NF-KB: Nuclear factor kappa B; AP-1: Activator protein-1; STAT-3: signal transducers and activators of transcription 3; SIRT-1: Sirtuin-1. (A color version of this figure is available in the online journal.)

MMP-9 secretion.[42] One of the *in vivo* studies using streptozotocin-induced diabetic rats showed that resveratrol favorably shifts ERK1/2 and p38 MAPK activation.[84] In contrast to all the above observations, Jiao et al. showed that treatment of glioblastoma-initiating cells with resveratrol although reduced Akt and mTOR phosphorylation, did not affect the phosphorylation of the ERK1/2, p38, and JNK pathways. The authors suggested that phosphoinositol-3-kinase (PI3K)/Akt but not the MAPK pathway were involved in resveratrol-induced inhibition of NF-κB activation.[37] The differential effect on two pathways observed in this study may perhaps be related to the concentration of resveratrol (10 and 20 μM) used in this study. Treatment of human cerebral microvascular endothelial cell line with resveratrol (0.1–10 μM) has been shown to cause activation of both the PI3K/Akt and ERK pathways leading to increased MMP-2 and MMP-9 secretion.[52]

Resveratrol activates SIRT1

MMPs' expression is also modulated by SIRT1. The deacetylation activity of SIRT1 regulates MMPs and is affected by several polyphenols. Intraperitoneal administration of resveratrol (10 mg/kg/day) in rats with streptozotocin-induced diabetes significantly activates SIRT1 gene expression in the heart tissue.[78] Treatment of human fibrosarcoma cell line with resveratrol at a concentration above 8 μM caused increased expression of SIRT1 and reduced expression of phosphoERK and MMP-9 at a concentration of 16 μM. Furthermore, treatment with resveratrol reduced expression of p65 and c-fos in the nucleus, which was indicative of the inhibition of nuclear translocation of NF-κB and AP-1, respectively.[38] PMA-induced activation of AP-1 in U937 cells has also been reported to be suppressed by resveratrol.[43] These results, therefore, suggest that resveratrol-induced inhibition of MMP-9 expression involves modulation of nuclear translocation of transcription factors such as NF-κB and AP-1, which in turn is affected by the total contents of SIRT1 intracellularly. Annabi et al., as described earlier, showed that preincubation of HBMEC with 30 μM resveratrol followed by a 30 min PMA led to diminished IκB phosphorylation. In the same study, it was observed that silencing of SIRT1 by transfecting cells with siRNA, designed to downregulate SIRT1, abolished the inhibitory effect of resveratrol on PMA-induced IκB phosphorylation.[40] Hence it could be concluded that SIRT1 is indeed important in the inhibitory potential of

resveratrol against PMA-mediated NF-κB signaling pathway. However, the inhibitory effect of resveratrol on MMP-9 persisted despite SIRT1 silencing, suggesting that effect of resveratrol on MMP-9 is SIRT1 independent. Resveratrol has also been shown to suppress the inflammatory responses of *P. gingivalis*-stimulated human gingival epithelial cells by inhibiting NF-κB signaling independent of SIRT1.[85] Lee et al. also showed that activation of SIRT1 in the presence of resveratrol inhibits the expression of MMP-9 in human fibrosarcoma cells through the suppression of reactive oxygen species, AP-1 and NF-κB activation by the enhanced activity of SIRT1.[38]

It is important to highlight that there is also antagonistic cross-talk between SIRT-1 and NF-κB signaling which implies that while SIRT-1 inhibits NF-κB; NF-κB down-regulates SIRT1 activity.[86] Hence, it is likely that the effects of resveratrol are not only dose-dependent but also context-dependent.

Resveratrol: challenges in translation from bench to bedside

There is minefield of data from preclinical studies regarding the biological activities of resveratrol. A Pubmed search with the key word ''resveratrol'' gives more than 8800 results. But search for ''resveratrol clinical trial'' limits the number of search results to 360. Understandably, there are multiple obstacles at present that have limited the progression of resveratrol as a therapeutic agent from bench to bedside. For instance, it remains unclear whether resveratrol itself or its metabolites exert the real biological effects. Resveratrol rapidly metabolizes and its monosulfates have been shown to possess significant biological activity.[87] However, studies have also shown that monosulfates may be converted back to resveratrol intracellularly.[88] Hence, it is likely that after cellular delivery, monosulfates are recycled back to resveratrol, which then produces the biological effects. Development of appropriate formulations is another obstacle currently. Development of prodrugs or carrier-based formulations may help in targeted delivery of resveratrol. Development of nanoformulations of resveratrol has been described widely; however, their detailed pharmacokinetic studies have not been done.[89] The issue of appropriate oral dose of resveratrol also needs to be investigated. In healthy volunteers, 5 g of resveratrol as single or multiple doses was found to be free of adverse effects. However, if this is true in patients with various conditions, such as cancer, remains unclear.[90] Understanding of the pharmacodynamics and precise mechanisms of action of resveratrol in various pathologies remains extremely complicated. It has multiple targets which seem to vary with the type of organ system involved, type of pathology targeted as well as the dose. It is important to determine the precise mechanisms of action to understand the scope of efficacy, optimum dose, duration of treatment, pharmacodynamics-related adverse effects as well as the appropriate patient population suitable to achieve therapeutic benefits of resveratrol. Interactions of resveratrol with other drugs and food are another area for investigations.

Conclusions

Disturbances of ECM homeostasis play a significant role in several pathological conditions besides cancer and its metastatic spread. Hence, it seems an appropriate approach to design therapeutic modalities that can normalize the ECM homeostasis. Due to significant effects of resveratrol on ECM deposition as well as its breakdown, its potential benefits have extensively been investigated in a wide range of diseases. It inhibits ECM deposition by suppressing the growth factors and their signaling pathways. However, the effects have largely been studied on TGF-β. It remains to be seen if other growth factors are also affected by resveratrol. Moreover, it remains to be determined if resveratrol could directly target the intracellular signaling pathways. The effects of resveratrol on MMPs are more variable. Some of the studies have shown that resveratrol increases MMP secretion while others have shown that it reduces MMP secretion. Similarly, the effects of resveratrol on molecular pathways such as those involving NF- κB and SIRT-1 vary from one study to the other. It is likely that factors related to experimental models, experimental environment, and the doses, perhaps collectively, trigger the molecular pathways in one or the other direction. Despite encouraging results from preclinical and some clinical studies, there are several challenges in translating these results into real clinical applications. Nevertheless, considering the wide ranging biological effects of resveratrol and absence of significant adverse effects even at high doses, resveratrol remains a potential candidate for translation from bench to bedside.

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DECLARATION OF CONFLICTING INTERESTS

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