Chapter 12
Grape Chemopreventive Agents Against Angiogenesis and Metastasis

Chandra K. Singh, Gagan Chhabra, Charlotte A. Mintie, and Nihal Ahmad

Abstract Recent advances in the identification of efficient diagnostic means, novel targets and therapeutics, have resulted in a significant decline in cancer mortality. However, based on recent estimates from the American Cancer Society, 1,762,450 new cancer cases and 606,880 cancer-deaths are projected to occur in the United States, in the year 2019. Therefore, an expedition for more efficient means of cancer management continues. Cancer cells cannot prosper without an adequate supply of blood through angiogenesis, a process of forming new blood vessels. Angiogenesis is a multistep process controlled by several proangiogenic and antiangiogenic factors. Dysregulated angiogenesis contributes to unlimited growth and metastasis of cancer, with fatal consequences. In a quest for novel agents/drugs to curtail metastatic spread of cancer, the dietary agents are being actively investigated. Grapes are, arguably, one of the most valuable fruits, containing more than 1600 phytochemicals. Among these, resveratrol, catechin, epicatechin, peonidin, cyanidin, malvidin, kaempferol, isorhamnetin, taxifolin, and quercetin are the top ten compounds that account for more than 70% of the grape polyphenols. These grape constituents alone, in combinations, or as whole grape products, have been shown to have anticancer activities. In this chapter, we have discussed the mechanistic action(s) of grape agents against angiogenesis and metastasis, both of which are crucial requirements for cancer survival and progression. Studies have shown how grape chemopreventive agents are proficient at challenging the proangiogenic and antiangiogenic factors necessary for tumor angiogenesis and metastasis. This makes grape antioxidants very promising in cancer management.

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12.1 Introduction

Angiogenesis and metastasis are linked processes involved in cancer progression and expansion. Angiogenesis is a multistep process promoting the growth of blood vessels from the existing vasculature that has been considered essential for tumor cell proliferation and viability, and represents as an essential component of the metastatic spread of the tumor. Angiogenesis is controlled by several proangiogenic and antiangiogenic factors and is considered to be crucial in the growth and progression of most tumors (Hanahan and Weinberg 2000, 2011). Distant organ metastasis, which is dependent on angiogenesis, is the major reason for cancer-fatalities. The word 'metastasis' stems from a Greek word meaning 'displacement', and represents a process when cancerous cells move from the primary tumor site (where the cells initially transformed) to other distant organs of the body. Metastasis is also a multistep process encompassing several inefficient steps including, (1) penetration of tumor cells deeper into surrounding tissues, (2) migration into the vessels, also known as 'intravasation', and (3) survival of the motile cells into the circulatory system and to distant organs where they can colonize and develop into a secondary lesion (Hanahan and Weinberg 2000, 2011; van Zijl et al. 2011). The capability of cancer cells to conquer and metastasize is extraordinarily complicated and one of the strategic symbols of cancer progression. In fact, primary carcinomas (except for lung and liver) hardly cause patient death, whereas most of the cancer-related mortality occurs due to complications linked to metastasis (van Zijl et al. 2011). The beginning of tumor metastasis includes invasion, which is facilitated by epithelial-mesenchymal transition (EMT), an event through which epithelial cells lose their cell-cell adherence and gain mesenchymal characteristics, resulting in increased migration potential and invasiveness of cancer. Hence, tumor angiogenesis and metastasis have been considered as pivotal points in tumor progression (Hanahan and Weinberg 2000, 2011; Singh et al. 2018), and thus, blocking these two phenomena of cancer could be useful in cancer management. Figure 12.1 outlines the key factors involved in tumor angiogenesis and metastasis.

As angiogenesis is one of the required critical events for cancer metastasis, it has been found that angiogenesis inhibitors would, therefore, avert or halt the growth of tumors. Several drugs are being used in the clinic that targets angiogenesis and metastasis. The Food and Drug Administration (FDA) has approved several antiangiogenic agents including monoclonal antibodies, proteasome inhibitors, tyrosine kinase inhibitors (TKIs) and mTOR inhibitors, such as Bevacizumab (Avastin), Bortezomib (Velcade), Gefitinib and Temsirolimus, respectively (Feng et al. 2010; Samant and Shevde 2011). The first case of successful antiangiogenic treatment was

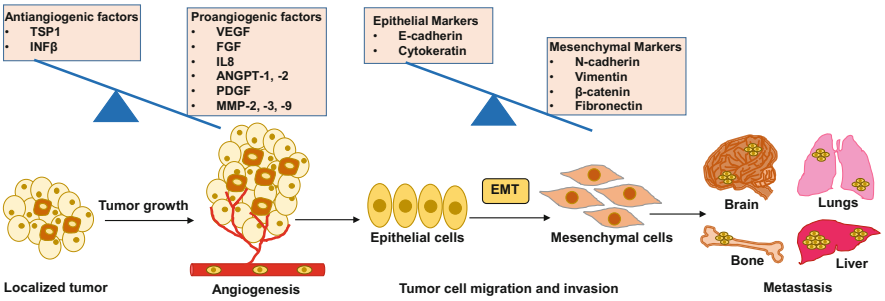

Fig. 12.1 Schematic diagram showing factors involved in tumor angiogenesis and metastasis

reported in 1989 when the drug interferon alfa-2a, a recombinant interferon possessing antiviral, immunomodulatory and antitumor characteristics, was used to suppress angiogenesis. This drug suppressed angiogenesis by inhibiting the production of two key angiogenic factors vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) (Feng et al. 2010). A monoclonal antibody that has been approved to curtail angiogenesis is Bevacizumab, which was approved in 2004 as a VEGF blocker to treat metastatic colorectal cancer, ovarian cancer, and breast cancer (Samant and Shevde 2011). TKIs, such as Sorafenib, Sunitinib, Gefitinib, and Erlotinib have also been developed to treat cancer by blocking angiogenesis pathways (Feng et al. 2010). However, these drugs present some unwanted adverse effects that may complicate the treatment. For example, although Bevacizumab has been shown to increase the survival of cancer patients, it is also associated with risks of diarrhea, hypertension, leucopenia, thrombosis, fatal hemorrhage and visceral perforation (reviewed in Sagar et al. 2006). In some cases, during a course of radiotherapy, tumors increase their angiogenic activity (Ansiaux et al. 2005). Resistance to antiangiogenic therapy also occurs frequently and constitutes a critical barrier in the inhibition of tumor growth. Additionally, the efficacy of antiangiogenic compounds varies from one tumor to another. If the angiogenic activity of a tumor is initiated primarily by only one or two factors, then blocking the activity of one factor may be enough to inhibit tumor growth. For example, expressions of VEGF and epidermal growth factor receptor (EGFR) correlate with the metastatic characteristics of human colon cancer, and therefore, targeting VEGF or EGFR may be beneficial (Iqbal and Lenz 2004). However, if several factors mediate the angiogenic activity in a particular tumor, additional intervention strategy may be required. Interestingly, grapes contain a range of compounds that may have synergistic or additive anti-proliferative activity against cancer (Singh et al. 2015a, 2016). Because of their pleiotropic mechanistic actions, these grape antioxidants may inhibit angiogenesis by interacting with multiple pathways and by affecting cell signaling that modulates other relevant processes such as apoptosis and the interaction of cells with the immune system. This chapter is dedicated to discussing the action(s) of grape compounds on mechanistic pathways relevant to angiogenesis and metastasis.

12.2 Tumor Angiogenesis

In order to understand how the process of angiogenesis occurs, it is essential to describe the origin of blood vessels, as they are pivotal in understanding this process. The cardiovascular system is one of the preliminary systems that develop in the embryo and is needed for supporting the nutritional requirements. Within the cardiovascular system are the endothelial cells, lining the surfaces of vascular structures (Risau 1997). These vascular endothelial cells play an important role in various processes such as tissue homeostasis, blood-tissue exchange, and blood cell activation. The vascular system forms by two processes, angiogenesis and vasculogenesis. Vasculogenesis is the formation of new vessels, whereas angiogenesis is the growth of vessels from pre-existing vessels. Angiogenesis begins during embryo formation and is necessary for the normal growth of embryonic and postnatal tissues, as well as for wound healing. It also contributes to the sprouting of vessels in organs that are avascular (i.e., kidney) and remodeling of the capillary networks to form smaller and larger vessels, as well (Risau 1997).

However, during cancer progression, cancer cells hijack this process for their growth. Unlike normal blood vessels, the blood vessels formed within tumors are dilated with uneven shapes. The process of tumor angiogenesis was first noticed by Judah Folkman, who hypothesized and demonstrated in 1971 that tumor growth is angiogenesis-dependent (Folkman 1971). Realizing that understanding the mechanism(s) of angiogenesis could lead to cancer therapies enthused intensive research in this field, as can be appreciated that there are over 55,000 manuscripts dealing with angiogenesis in cancer research (PubMed search; keywords 'angiogenesis and cancer'; June 2019). Cancer cells instigate angiogenesis quite early during the development of a tumor. The angiogenesis is characterized by oncogene-driven tumor expression of pro-angiogenic proteins. Angiogenesis plays a crucial task in the progression of most solid tumors, including those of bladder, brain, breast, cervix, colon, lung, and prostate. An increasing density of tumor vasculature raises the probability that the tumor will metastasize (Bielenberg and Zetter 2015).

Although the process of angiogenesis is quite complex, one critical growth factor very important in this process is the vascular endothelial growth factor (VEGF) (Carmeliet and Jain 2011). VEGF is one of the most well-studied growth factors involved in angiogenesis. With the loss of a single VEGF allele, there is a link to embryonic vascular defects (Carmeliet 2003). Also known as VEGF-A, VEGF stimulates the process of angiogenesis by signaling through another component VEGF receptor-2 (VEGFR2) (Lee et al. 2015b). A high level of VEGF is associated with poor disease outcome in a wide array of malignancies. VEGF accelerates the proliferation and migration of endothelial cells and stimulates the levels of plasminogen and metalloproteinases. In several animal models, overexpression of VEGF in tumor cells enhances tumor growth and metastasis by stimulating vascularization (Risau 1997). Expression of VEGF mRNA is upregulated by many oncogenes (including H-RAS, K-RAS, SRC, and C-JUN) and growth factors [including epidermal growth factor (EGF), transforming growth factor (TGF)-α and -β, insulin-like

growth factor-1 (IGF1), and platelet-derived growth factors (PDGF) (Hanahan and Weinberg 2000; Lee et al. 2015b)].

Another key group of molecules that are important in angiogenesis is the PDGF family of proteins, which consists of TGF-β and angiopoietins. TGF-β has shown to be an important signaling pathway in angiogenesis. This family of molecules consists of several structurally similar growth factors that play important roles in embryonic and postnatal angiogenesis. The PDGF signaling pathway has some parallels to the VEGF system (Thurston and Daly 2012). Angiopoietins (ANGPTs), a family of four proteins, are extracellular ligands that bind to endothelial cell-specific tyrosine kinase receptor TIE2 (Fagiani and Christofori 2013). Two of these, ANGPT1 and ANGPT2, are well studied and described for its role in angiogenesis. ANGPT1 is known to be crucial for vessel growth, adhesion, migration, and survival. ANGPT2 is generally limited to endothelial cells and known to stimulate cell death and disrupts vascularization. However, in conjunction with VEGF, ANGPT2 supports neo-vascularization, suggesting that it may be useful together with anti-VEGF treatments against cancer (Fagiani and Christofori 2013).

Fibroblast growth factor (FGF) family has also been shown to play a pivotal role in the process of angiogenesis. In fact, basic fibroblast growth factor (bFGF) also known as FGF2 is one of the first angiogenic factors to be discovered (Carmeliet and Jain 2011). Another commonly studied form of FGF is acidic fibroblast growth factor (aFGF), also known as FGF1. FGF1 and FGF2, like other FGF family members, possess mitogenic and cell survival activities, and play a crucial role in tumor growth and invasion (Beenken and Mohammadi 2009). FGFs are known to stimulate fibroblast growth factor receptors (FGFRs) on endothelial cells as well as trigger angiogenesis by producing angiogenic factors from other cell types. In tumors, aberrant FGF signaling has been noted to promote angiogenesis. However, the development of anti-FGF treatments are lagging as 22 members FGF family are structurally related and show biologically substantial redundancy (Beenken and Mohammadi 2009; Ucuzian et al. 2010).

During the process of wound healing, the eruption of angiogenesis must be stopped once the newly developed capillaries have reached a certain density. Therefore, a homeostatic equilibration of proangiogenic and antiangiogenic factors is very crucial for normal cells. The development and progression of many cancers are associated with lack of the endogenous angiogenesis inhibitors. For example, a potent inhibitor of angiogenesis, interferon beta (INFβ) works by blocking interleukin-8 (IL8) (Oliveira et al. 1992), bFGF (Singh et al. 1995) and collagenase type V (Gohji et al. 1994), which all are angiogenic factors aiding in tumor development and invasiveness. Tumor cells also halt production of angiogenesis inhibitor thrombospondin-1 (TSP1), which is regulated by tumor suppressor protein P53 (Grant et al. 1998). Interestingly, P53 loss is frequently noticed in more than 50% of the cancers. Currently, antiangiogenic therapies are being used to manage malignancies, while proangiogenic therapies are being investigated to handle cardiovascular diseases. Some of the angiogenic factors and their regulation is shown in Figs. 12.1 and 12.2 (Bashir et al. 2002; Hanahan and Weinberg 2000, 2011).

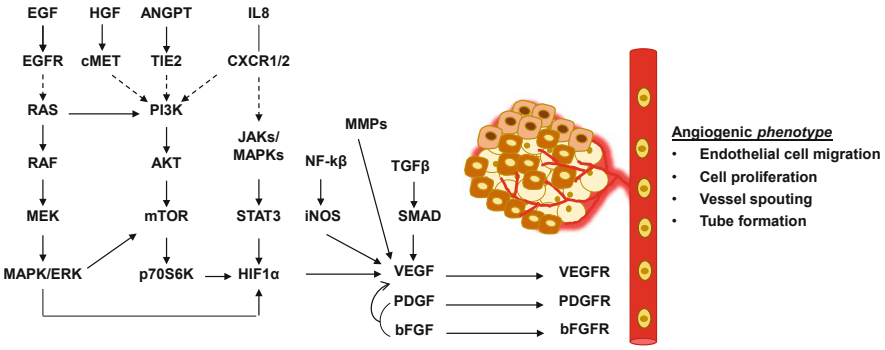

Fig. 12.2 Key mediators involved in tumor angiogenesis

12.3 Tumor Invasion and Metastasis

Tumor invasion is the direct extension and penetration of cancer cells into adjacent tissues, while metastasis is the expansion of cancer cells through the circulatory or lymphatic system to distant sites. Metastasis is of crucial importance in cancer progression because most cancer-related deaths are due to metastatic spread to cancer cells affecting organs secondary to original tumor locale (Hanahan and Weinberg 2000, 2011; van Zijl et al. 2011). Commonly, tumor metastases occur in lungs, liver, brain, and bones after localization to the lymph nodes. Metastasis is an important factor in deciding the staging of cancer, depending on the extent to which cancer has spread. Interestingly, metastatic tumors shed ~1 million cancer cells per gram of tumor in the blood circulation each day, and out of them, ~0.01% cells possess the characteristics of metastatic progenitor cells, which is also studied as one of the key targets for metastatic prevention (Chang et al. 2000; Langley and Fidler 2011; Payen et al. 2017).

Epithelial-mesenchymal transition (EMT) has been progressively recognized as a key event in tumor metastasis. EMT is characterized by loss of epithelial markers (E-) cadherin (ECAD), which is a crucial component of adherence junctions, and cytokeratin (CK1), which is a key component of intermediate filaments. Loss of ECAD by the transcriptional repressors SNAIL, SLUG, ZEB2 or TWIST leads to the dismantling of adherence junctions and translocation of membrane-bound β-catenin (CTNNB) to the cell nucleus (Thiery et al. 2009; van Zijl et al. 2011). EMT is also characterized by concurrent gain of mesenchymal markers such as vimentin (VIM) and N-cadherin (NCAD). This change from E- to N-cadherin expression, termed cadherin-switch, leads to enhanced motility of EMT-transformed cells (van Zijl et al. 2011; Yilmaz and Christofori 2010). The molecular machinery of EMT process has been a target of anti-cancer drug development as well as for their potential use as biomarkers of cancer progression.

The molecular mechanism of metastasis involves several critical molecular events. The genes responsible for metastasis are mostly developmentally non-essential stress response genes that, if expressed, physiologically facilitate the homing of immune cells. Two key proteins involved in this progression are CD44, a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion, and migration, and osteopontin (OPN), which facilitates the linking of osteoclasts to the mineralized bone matrix (reviewed in Weber 2008). CD44 is expressed on macrophages and lymphocytes and contains ten exons that are prone to alternative splicing. The presence of CD44, though not essential for early transformation and growth, has been demonstrated to be a key regulator of metastasis (Weber 2008; Weber et al. 2002). Recent studies have shown the characterization of two isoforms of CD44, which are standard CD44 (CD44s) and variant CD44 (CD44v). CD44v overexpressed in metastasized tumors, and the interplay of CD44v and CD44s has been suggested to play a role in regulating EMT (reviewed in Chen et al. 2018a). The crucial other protein, OPN, is essential in macrophage filtration during stimuli responses. Interestingly, macrophages in the tumor microenvironment have been suggested to promote tumor invasion and metastasis via epidermal growth factor (EGF) and EGF receptor (EGFR). Additionally, OPN is overexpressed in several cancers and has been correlated with poor prognosis in patients (Anborgh et al. 2010). OPN is also a mediator that protects against pathogens. Both CD44 and OPN, are stress response proteins known to be involved in physiological defenses (Weber 2008). The manipulation of plasma or tumor tissue-specific levels of CD44 and OPN has been suggested as a useful strategy to manage cancer metastasis. Figure 12.3 outlines the major signaling mediators involved in EMT and metastasis.

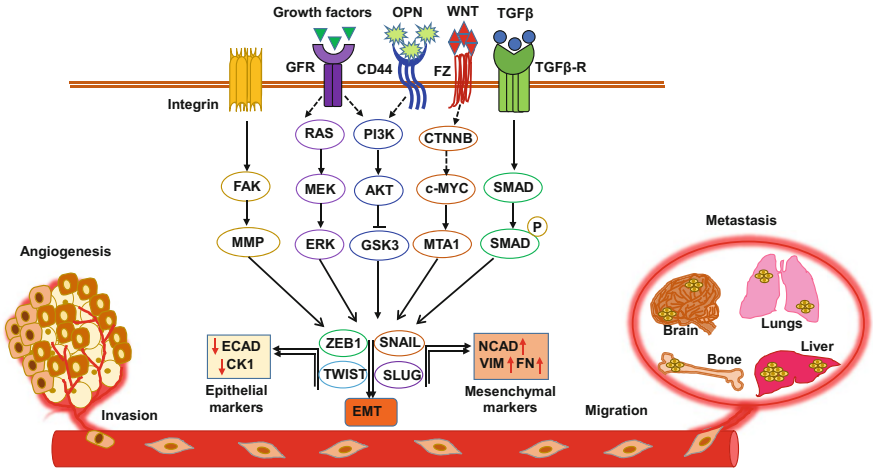

Fig. 12.3 Signaling mediators involved in EMT and metastasis

12.4 Grape Antioxidants Against Angiogenesis and Metastasis

Historically, plant-derived agents (including herbs, fruits and vegetable, and their individual constituents) have been widely investigated and used for achieving health benefits and in the management of a variety of disease conditions (Greenwell and Rahman 2015). Grape (*Vitis vinifera*) is probably one of the most valuable fruits, in terms of availability of health-beneficial phytochemical constituents. In two recent publications, we have discussed how grape constituents, in its natural combination in whole grape or in certain specific combination may be useful in the management of diseases including cancer (Singh et al. 2015a, 2016). Grapes are very rich in antioxidants and contain a number of phytochemicals including stilbenes, phenolic acids, and flavonoids. The actual composition of these phytochemicals varies greatly among different grape varieties. The medicinal use of grape/grape constituents dates back to ancient time. For example, traditional Ayurvedic tonic called Drakshasava, which is very popular in the Indian subcontinent is made from fermented grapes. Drakshasava is used to manage a variety of health issues including lethargy, weakness, and heart-health. Chromatographic assessment of Drakshasava established the presence of numerous polyphenols, including resveratrol and pterostilbenes (Paul et al. 1999). In the early 1990s, red wine derived from grapes gained popularity as a reason for the "French Paradox", which accounts for the somewhat paradoxical epidemiological observation that French population have a relatively lower risk of certain heart diseases despite the high-fat diet, owing to the consumption of red wine (Renaud and de Lorgeril 1992). Since then, hundreds of compounds have been identified in grapes that have been linked with a variety of health benefits (Pezzuto 2008). As discussed in a recent study from our laboratory, where we assessed the effect of grape powder against UVB-mediated skin carcinogenesis in SKH-1 hairless mice, the top ten antioxidants of the grape powder are resveratrol, catechin, epicatechin, peonidin, cyanidin, malvidin, kaempferol, isorhamnetin, taxifolin, and quercetin (Singh et al. 2019). Individually, these agents, are widely reported for their anticancer activities. Here, we have discussed the studies suggesting that these grape-based individual chemopreventive agents alone or, in combinations, including extracts, are promising against angiogenesis and metastasis.

12.4.1 Grape Stilbene Resveratrol

Resveratrol, a well-known grape antioxidant, is a phytoalexin produced by plants to prevent parasitic growth. Chemically, resveratrol (3,5,4′-trihydroxystilbene), a stilbenoid that exists in *trans*- and *cis*-configuration. The *trans*-resveratrol is known to photo-isomerizes to cis-resveratrol in the presence of ultraviolet irradiation (Figueiras et al. 2011). Resveratrol has been characterized as one of the most

important active agents of the grape powder (0.69–1.01 mg/kg) (Singh et al. 2019) and grape-derived products such as red wine (concentrations of 0.1–14.3 mg/L) (Mukherjee et al. 2010). In fact, the resveratrol in red wine has been touted as a major reason behind the so-called *French Paradox* (Catalgol et al. 2012). Though bioavailability of resveratrol is somewhat low (~0.5%) due to immediate hepatic glucuronidation and sulfation (Walle et al. 2004), it has been shown to elicit broader biological effects against various health conditions including cancer, aging, diabetes, cardiovascular diseases, etc. (Singh et al. 2015b). This has been explained because of a number of reasons; including the regeneration of resveratrol from an intracellular pool of resveratrol sulfates (Patel et al. 2013), and the fact that some resveratrol metabolites like resveratrol 3-sulfate are known to possess their own biological activity (Hoshino et al. 2010).

The effect of resveratrol against cancer in multiple animal models has shown to be mostly protective with some exceptions of no effect depending on cancer types and resveratrol dose and route of administration. Similar results have been seen in the clinical trials, except one case where some adverse events were noticed in multiple myeloma patients (Berman et al. 2017; Popat et al. 2013). Interestingly, no adverse events were found when the same formulation was used in other studies (Popat et al. 2013; Singh et al. 2015b). In the recent past, we have reviewed the issues and questions which hinder the clinical translation of resveratrol for cancer management (Singh et al. 2015b). To understand the challenges that need to be overcome for the success of resveratrol to the clinic, it's important to examine the key signaling molecules/pathways known to be affected by resveratrol in cancer. The effect of resveratrol against key cancer signaling molecules/pathways is well documented in multiple cell culture experiments and animal studies. For example, resveratrol has been found to inhibit key cancer-promoting pathways, e.g., IGF-1R/AKT/WNT, PI3K/AKT/mTOR and NF-κB signaling pathways, which are known to be associated with tumor growth and progression (reviewed in Berman et al. 2017).

The effect of resveratrol against angiogenesis has been widely studied. Resveratrol has been found to inhibit angiogenesis and tumor growth in human breast cancer xenografts as well as reduce levels of VEGF in MDA-MB-231 breast cancer cells (Garvin et al. 2006). Yu et al. have shown the inhibitory effect of resveratrol on VEGF and angiogenesis in hepatocellular carcinoma partly via inhibiting NF-κB signaling (Yu et al. 2010). Resveratrol has been found to inhibit tumor growth in rat RT-2 gliomas and angiogenesis in the glioma cells (Tseng et al. 2004). Resveratrol-mediated inhibition of rat glioma has been shown to be associated with the suppression of macroscopic and microscopic angiogenesis (Chen et al. 2006). Hu et al. have found modulation of several important angiogenic factors such as VEGF, bFGF, matrix metallopeptidase-2 and -9 (MMP2 and MMP9) in exerting the antimyeloma effects of resveratrol (Hu et al. 2007). The antiangiogenic effect of resveratrol in human ovarian cancer cells has shown to be mediated via inhibition of VEGF as well as hypoxia-inducible factor 1α (HIF1α), which plays a key role in tumor progression (Cao et al. 2004; Park et al. 2007). The antiangiogenic activity of resveratrol has also been demonstrated by modulation of TSP1, which is a downstream target of P53 and known to inhibit angiogenesis naturally. In this study, Trapp et al. have shown the

correlation of antiangiogenic effects of resveratrol with increased P53 and TSP1, and decreased HIF1α and VEGF levels (Trapp et al. 2010).

Similarly, resveratrol's antimetastatic potential has been demonstrated in various model systems. Wu et al. have investigated the antimetastatic potential of resveratrol under normoxia and hypoxia conditions, and found that resveratrol restricts the cell migration, adhesion, and invasion in colon carcinoma cells. This was mediated by a reduced level of VEGF, MMP2, and MMP9 under normoxia and hypoxia, and reduced HIF1α under hypoxia (Wu et al. 2008). Resveratrol has also been shown to modulate key EMT-related markers that are crucial for cancer cell motility, invasiveness, and metastasis. Wang and colleagues have shown resveratrol as an inhibitor of TGF-β1-induced EMT, where resveratrol was found to inhibit cell adhesion, migration, and invasion of A549 lung cancer cells. In this study, resveratrol was found to increase epithelial marker ECAD and represses mesenchymal markers, fibronectin (FN) and VIM, inhibits expression of EMT-inducing transcription factors SNAIL and SLUG (Wang et al. 2013). Resveratrol has also been shown to inhibit characteristics of pancreatic cancer stem cells (CSCs) originated from human primary tumors as well as in KRAS(G12D) transgenic mice by inhibiting self-renewal capacity and EMT transcriptional regulators ZEB1, SLUG, and SNAIL (Shankar et al. 2011). Resveratrol treatment of human tongue squamous cell carcinoma cell line CAL-27 showed decreased cell migration, invasion by inhibiting the EMT-inducing transcription factors (Kim et al. 2018). Resveratrol has been shown to inhibit invasion and metastasis in gastric cancer cells via inhibiting hedgehog signaling and EMT (Gao et al. 2015). In a study by Sheth et al., resveratrol administration has been found to inhibit prostate tumor growth as well as lung metastasis by inhibiting the AKT/MicroRNA-21 pathway (Sheth et al. 2012). Figure 12.4 outlines the molecular actions of resveratrol against angiogenesis and metastasis.

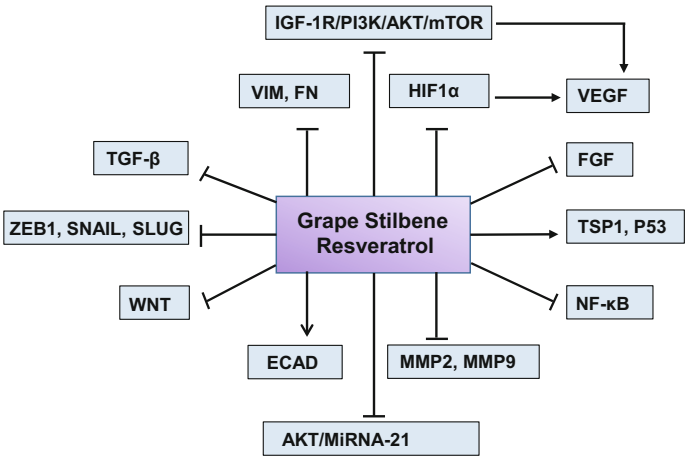

Fig. 12.4 Molecular actions of resveratrol against angiogenesis and metastasis

12.4.2 Grape Flavonols (Quercetin, Kaempferol, Isorhamnetin, Taxifolin)

Flavonols are a class of flavonoids that are known to act as antioxidants to reduce oxidative stress and also can serve as anti-inflammatory agents (Cook and Samman 1996). Flavonols are known to inhibit CYP2C9 and CYP3A4 enzymes, which metabolize most drugs in the body (Sprouse and van Breemen 2016). Although many flavonols are present in dietary grape, the four most common are quercetin, kaempferol, isorhamnetin and taxifolin (Singh et al. 2019), all of which have been individually evaluated among many diseases and disorders demonstrating beneficial effects.

Quercetin (chemically, 5,7,3′,4′-flavon-3-ol) has been shown to prevent low-density lipoprotein from oxidizing, modify eicosanoid biosynthesis (anti-inflammatory responses), prevent platelet accumulation, and promote relaxation of cardiac smooth muscle (Formica and Regelson 1995). Quercetin is a powerful antioxidant, which has been shown to have potential in cancer management. Quercetin possesses anti-inflammatory functions, which are mainly attributed to its inhibitory effect on inflammatory mediators like nitric oxide, catalase, and pro-inflammatory cytokines (Ward et al. 2018). In addition, quercetin exerts an apoptotic effect in tumor cells and can block cancer progression. Quercetin has demonstrated cancer chemopreventive effects in multiple models (Gibellini et al. 2011). Quercetin has been shown to inhibit angiogenesis in several models through multiple mechanisms, including interaction with the cyclooxygenase-2 (COX2) and lipoxygenase-5 enzymes, EGFR, the HER2 intracellular signaling pathway, and the NF-κB nuclear transcription protein (reviewed in Sagar et al. 2006). In ascite cells of Dalton's lymphoma-bearing mice, quercetin attenuated levels of VEGF-A, COX2, iNOS and NO (Maurya and Vinayak 2017). The treatment of breast cancer cells with gold nanoparticles carrying quercetin demonstrated inhibited angiogenesis, EMT, and metastasis of mammary tumors in Sprague-Dawley rats (Balakrishnan et al. 2016). Treatment with the quercetin inhibited EGFR-mediated AKT signaling and reduced migration in human pancreatic cells and human head and neck squamous cell carcinomas (Chan et al. 2016; Lee et al. 2015a). In oral cancer, quercetin-induced growth inhibition has been found to be associated with inhibition of EGFR/AKT activation with simultaneous activation of Forkhead Box O1 (FOXO1) (Huang et al. 2013), which is one of four members of FOXO transcription factors and known to inhibit angiogenesis in certain cancers (Kim et al. 2016).

Quercetin also demonstrates the ability to inhibit angiogenesis in prostate cancer through the VEGFR2 pathway, by regulation of the AKT/mTOR/P70S6K signaling pathways both in vitro and in vivo (Pratheeshkumar et al. 2012). Quercetin has been shown to prevent EGF-induced EMT via inhibition of EGFR/PI3K/AKT/ERK1/2 pathway as well as by suppressing transcriptional repressors SNAIL, SLUG and TWIST in prostate cancer cells. As per this pathway, quercetin may prevent cancer metastasis by targeting EMT (Bhat et al. 2014). Additionally, in prostate cancer, the anticancer effects of quercetin combined with tamoxifen enhanced antiangiogenesis effects (Ma et al. 2004). A study by Igura et al. examining the effects of resveratrol

and quercetin on angiogenesis found decreased growth of bovine aorta endothelial (BAE) cells in a concentration-dependent manner in vitro. The migration of BAE was substantially inhibited by resveratrol, but only weakly inhibited by quercetin (Igura et al. 2001).

Another potentially therapeutic flavonal, kaempferol (chemically 3,4′,5,7-tetrahydroxyflavone), is known to act as modulators of EMT markers (NCAD, ECAD, SLUG, and SNAIL) and metastasis marker MMP2 (reviewed in Imran et al. 2019). In ovarian cancer cell lines, kaempferol works against angiogenesis through multiple pathways including the HIF-dependent (AKT/HIF) and HIF-independent estrogen-related receptor alpha (ESRRA) pathways, (Luo et al. 2009) and by impairing VEGF synthesis through the ERK/NF-κB/cMYC/P21 pathway (Luo et al. 2012). Reduction of VEGF secretion was also noted in human MDA breast cancer cells upon kaempferol treatment (Schindler and Mentlein 2006). Another breast cancer study demonstrated that kaempferol treatment did not affect secretion, but did significantly inhibit the activity of MMP3 accompanied by a blockage of MDA-MB-231 cell migration (Phromnoi et al. 2009). Reduced migratory effects in prostate cancer cells after kaempferol treatment was suggested to be due to the inhibitory effects of kaempferol on EGFR, and further SRC, AKT and ERK related survival, migration, and invasion (Lee and Kim 2016). Medulloblastoma cell line migration was also decreased upon treatment with kaempferol or quercetin, suggestively through a reduction of hepatocyte growth factor (HGF)-mediated activation of AKT (Labbé et al. 2009).

Limited information is available regarding the potential anti-cancer effects of grape flavonols isorhamnetin and taxifolin. Isorhamnetin is a 3′-methoxylated derivative of quercetin and known for its anticancer properties demonstrated in certain cancers. For example, isorhamnetin has shown to suppress colon cancer cell growth by inhibiting the PI3K/AKT/mTOR pathway (Li et al. 2014). Likewise, taxifolin (5,7,3′,4′-flavan-on-ol), also identified as dihydroquercetin, has been suggested to exert chemopreventive activity by modulating antioxidant response element (ARE) mediated gene regulation (Lee et al. 2007). Although there is a general lack of studies of these two flavonols, implications of the antiangiogenic and antimigratory effects suggest the potential basis of anti-cancer effects of these agents. In a Lewis lung cancer mouse model, isorhamnetin treatment reduced VEGF protein expression, metastatic lesions, and tumor cell density. The results were enhanced further when isorhamnetin was combined with cisplatin (Zhu et al. 2017). In MDA breast carcinoma cells, the downregulation of MMP2 and MMP9 suggest that isorhamnetin may act to suppress p38 MAPK and STAT3 to inhibit invasion (Li et al. 2015). Isorhamnetin also reduced EMT and pulmonary fibrosis in a murine model (Zheng et al. 2019). Similar to other grape flavonols, taxifolin reduces AKT, as well as represses cell migration and invasion of osteosarcoma cells (Chen et al. 2018b). In skin scar cell carcinoma (SSCC), the inhibited invasion SSCC cells were associated with downregulation of MMP2 and MMP9 by taxifolin (Zhou et al. 2019). However, taxifolin, compared to kaempferol and quercetin, does not possess a strong cytotoxic effect in reducing VEGF in human ovarian cancer cells (Luo et al. 2008). Figure 12.5 outlines the molecular actions of grape flavonols against angiogenesis and metastasis.

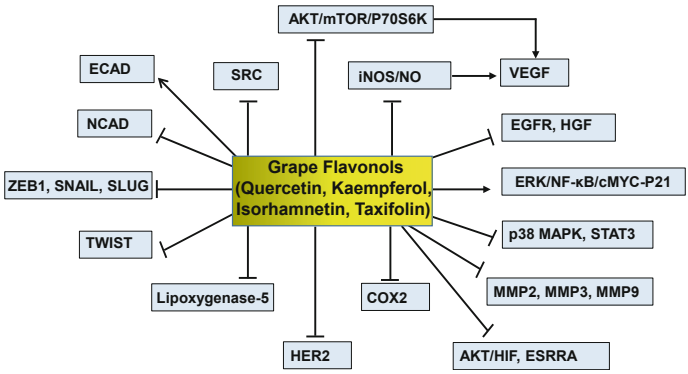

Fig. 12.5 Molecular actions of grape flavonols against angiogenesis and metastasis

12.4.3 Grape Catechins (Catechin, Epicatechin)

Catechin and epicatechin have been quantified as two major components of the top ten compounds of the grape powder (Singh et al. 2019). These are epimers, with D-catechin and L-epicatechin being the commonly occurring optical isomers in nature. Catechins fall into the flavonoid family and give rise to other oligomeric compounds including proanthocyanidins (or condensed procyanidins) that yield cyanidin when depolymerized under oxidative conditions. Catechin is the major monomeric polyphenol in whole grape or its products such as red wine. In addition to other observed mechanisms of action that lead to an antiproliferative effect in various cancer, catechin has been shown to inhibit angiogenesis in certain cancers. Ebeler et al. have shown that dietary supplementation of catechin delayed tumor onset in a linear dose-dependent manner in a transgenic mice model of neurofibromatosis. It was also found that the plasma levels of catechin and its metabolite 3′-*O*-methylcatechin (3′MC) were positively associated with the delay in tumor onset, without obvious adverse effects in mice (Ebeler et al. 2002). In another study, Payen and colleagues demonstrated that catechins inhibited cancer cell migration in vitro in a model of super-invasive human cervix cancer cells SiHa-F3. Further, (+)-catechin: lysine complexes (1:2) were shown to prevent metastasis of B16F10 melanoma cells to the lungs of C57BL/6JRj male mice (Payen et al. 2017).

Grape seed extract (GSE) obtained from Palieri grape cultivar that contained 6.2 mg/g catechins and 5.6 mg/g procyanidins were assessed for their effects against MDA-MB231 breast cancer cell migration and invasion (Dinicola et al. 2014). GSE at a sub-lethal concentration (25 μg/ml) strongly inhibited cell migration and invasion ability of MDA-MB231 cells. This was accompanied by a decrease in urokinase-type plasminogen activator (uPA), MMP2 and MMP9 activities, as well as in a down-regulation of CTNNB, fascin (FSCN1) and NF-κB expression (Dinicola et al. 2014). Another study was performed on procyanidin B2-3,3″-di-*O*-gallate (B2G2), a component of grape seed extract, where it was shown to inhibit the motility and invasiveness of human umbilical vein endothelial cells (HUVECs) and

human prostate microvascular endothelial cells (HPMECs). Mechanistic studies have shown that B2G2 targets VEGFR2/PI3K/AKT and integrin signaling molecules, which are important for endothelial cells survival, proliferation, tube formation, and motility (Kumar et al. 2015). Overall, this study showed that B2G2 inhibited several attributes of angiogenesis in cell culture and warrants future studies for the efficacy of B2G2 for angio-prevention and cancer control (Kumar et al. 2015).

The grape seed proanthocyanidins (GSP) are composed of dimers, trimers, tetramers, and oligomers of monomeric catechins or epicatechins (Shi et al. 2003). Feng et al. (2014) have shown the effects of grape proanthocyanidins on tumor angiogenesis in liver cancer (hepatocellular carcinoma or HCC) xenograft models. In this study, they have shown that grape proanthocyanidins inhibited tumor cells growth and metastasis by exerting antiangiogenesis effects via inhibition of microvessel density (MVD), which is a quantitative index of tumor angiogenesis. It is related to the supply of nutrition and oxygen to tumors towards proliferation, invasion, growth, and metastasis of tumor cells (Zhao et al. 2006). MVD is calculated by labeling cells with an anti-CD34 antibody specific to vascular endothelial cells in the tumor tissues followed by counting the number of microvessels per unit area to reflect the degree of angiogenesis in tumor tissues. In this study, a gradual reduction in MVD was observed with increasing concentration of grape proanthocyanidins including a significant positive correlation between MVD and the expression of angiogenesis marker VEGF. Tumor angiogenesis involves various types of growth factors, out of which VEGF is the most prevailing and a potent inducer of capillary growth into a tumor. VEGF, a hexose-modified multifunctional protein, specifically acts on vascular endothelial cells, inducing micro-angiogenesis and causing tumor invasion and metastasis. It was also suggested that grape proanthocyanidins may exhibit the antiangiogenic activity by inhibition of vascular endothelial cell proliferation (Feng et al. 2014).

Further, grape seed proanthocyanidin extract containing 5000 ppm resveratrol (GSPE) has been shown to facilitate oxidant-induced VEGF expression in keratinocytes. Using a ribonuclease protection assay (RPA), GSPE has been shown to regulate oxidant-induced changes in several angiogenesis-related genes. Further, pretreatment of HaCaT keratinocytes with GSPE upregulated both hydrogen peroxide (H_2O_2) and TNFα-induced VEGF expression and release. The results of the study suggested that GSPE might have beneficial therapeutic effects in promoting dermal wound healing and other related skin disorders (Khanna et al. 2001). Similarly, Luan et al. investigated the anti-vasculogenic mimicry (VM) activity of grape seed proanthocyanidins in human triple negative breast cancer (TNBC). This study demonstrated that highly aggressive TNBC cells HCC1937 formed vasculogenic-like network structures when cultured on a three-dimensional matrix compared to aggressive MCF-7 cells that were unable to form the patterned networks with the same conditions. Interestingly, grape seed proanthocyanidins inhibited proliferation of HCC1937 cells significantly and suppressed tubular-like structures. Therefore, this study suggests that grape seed proanthocyanidins may be a potential anti-VM agent for human TNBC (Luan et al. 2015).

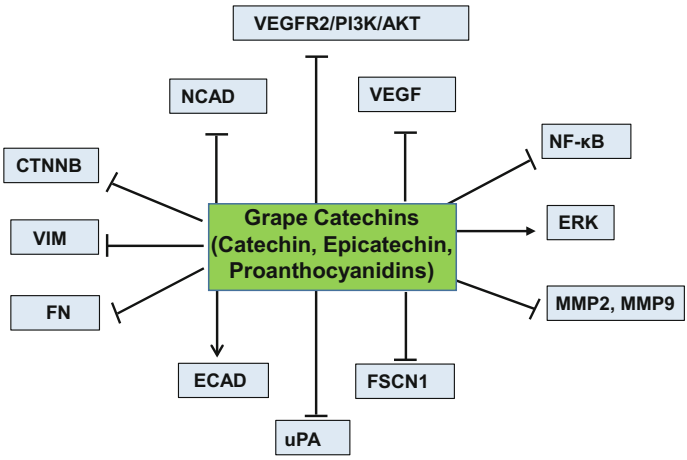

Fig. 12.6 Molecular actions of grape catechins against angiogenesis and metastasis

Further, Sun et al. assessed the chemotherapeutic effects of GSP on the invasion potential of human head and neck cutaneous squamous cell carcinoma (SCC) cells. The results obtained from this study suggested that GSP has the ability to inhibit the invasion of head and neck cutaneous SCC cells by targeting the EGFR expression and reversing the process of EMT (Sun et al. 2011). Further, GSP treatment of SCC13 cells showed the down-regulation of mesenchymal biomarkers, such as VIM, FN and NCAD while up-regulated the expression of the epithelial biomarker ECAD. These data suggested that inhibition of EMT by GSP may also be one of the possible mechanisms through which it reduces the invasiveness of cutaneous head and neck SCC cells and that lead to inhibition of invasion ability of SCC13 cells (Sun et al. 2011). Figure 12.6 shows the molecular actions of grape catechins against angiogenesis and metastasis.

12.4.4 Grape Anthocyanins (Cyanidin, Peonidin, Malvidin)

Anthocyanins belong to a group of flavonoids and are known for their antioxidant, anti-inflammatory and anticancer effects. Cyanidin, peonidin, and malvidin are the top thee anthocyanins characterized in grape powder (Singh et al. 2019) or, grape powder extract (van Breemen et al. 2016). Anthocyanins isolated from fruits of *Vitis coignetiae* Pulliat, a variety of wild grape called meoru in Korea and is used in Korean folk medicine, has been investigated against angiogenesis and metastasis. Compositional analysis of this anthocyanins identified the presence of the highest amount of cyanidin along with other anthocyanins peonidin, malvidin, delphinidin and petunidin (Lu et al. 2017). Lu et al. have demonstrated that the anthocyanins extract inhibited cancer cell proliferation, invasion, and angiogenesis in human lung

cancer A549 cells. In this study, anthocyanins have been shown to suppress migration and invasion of cancer cells by inhibiting MMP2 and MMP9 expression as well as adhesion and angiogenesis by inhibiting intercellular adhesion molecule 1 (ICAM1) and VEGF (Lu et al. 2017). In another study, Lu et al. investigated the effects of anthocyanins on cellular responses and molecular changes intricate in cancer invasion and EMT in EGF or TGF-β treated human lung cancer cells. This extracted anthocyanin was found to repress glycogen synthase kinase-3β (GSK3β) phosphorylation and CTNNB expression that is involved in EMT. This study demonstrated that anthocyanin inhibited PI3K/AKT and EGFR pathway independently in a dual repression mode as well as inhibited invasion and migration at least in part by suppressing EMT (Lu et al. 2014). Anthocyanins have also been shown to inhibit invasion and EMT markers in human uterine cervical cancer HeLa cells. Treatment with anthocyanins suppressed mesenchymal markers VIM, NCAD, and CTNNB expression and induced epithelial marker ECAD as well as suppressed expression of SNAIL, a transcriptional regulator of EMT (Lu et al. 2013). Furthermore, Burton and colleagues have shown that anthocyanin comprising muscadine grape skin extract inhibited SNAIL and pSTAT3, and abolished SNAIL-mediated cathepsin L (CTSL) activity, migration, invasion, and osteoclastogenesis in the breast (MCF-7) and prostate (LNCaP, ARCaP-E) cancer cells (Burton et al. 2015). In conclusion, these studies suggest that grape anthocyanins have antiangiogenesis and antimetastatic activities.

Further, there is limited literature available assessing the effects of anthocyanins individually against cancer, showing their anti-angiogenic and/or anti-metastatic potential. In one study, cyanidin-3-glucoside (C3G) was found to inhibit UVB-mediated oxidative damage and inflammation in SKH-1 hairless mice. Specifically, C3G inhibited glutathione depletion, lipid peroxidation, myelo-peroxidation and pro-inflammatory cytokines (IL6 and TNFα) in mouse skin. Further, C3G supplementation modulated UVB-induced MAP kinase and NF-κB signaling pathways (Pratheeshkumar et al. 2014). Liu and colleagues have shown that C3G, as well as peonidin-3-glucoside (P3G), inhibited the phosphorylation of human epidermal growth factor receptor-2 (HER2), which is known to be overexpressed in breast cancer as well as known to upregulate angiogenesis at different levels in cells (Alameddine et al. 2013; Liu et al. 2013). The antiproliferative effect of P3G and C3G were shown to be related to inhibited p-AKT and induced apoptosis in HER2-positive breast cancer cells (Liu et al. 2013). Moreover, in HER2-positive MDA-MB-453 xenografted mouse supplemented with P3G or, C3G, at a dose of 6 mg/kg, were found to reduce tumor growth (Liu et al. 2013). Importantly, in a clinical trial, assessing the bioavailability of isotopically labeled C3G (500 mg), has found relatively more bioavailable and metabolites were noticed in the circulation for $\leq$48 h after ingestion (Czank et al. 2013). Like cyanidin and peonidin, malvidin has been shown to inhibit TNFα-induced inflammatory response in endothelial cells, indicating its possible role in preventing atherosclerosis and cancer. Specifically, malvidin has been shown to inhibit TNFα-induced increase in monocyte chemotactic protein-1 (MCP1), vascular cell adhesion molecule-1 (VCAM1), intercellular adhesion molecule-1 (ICAM1), IκBα degradation and NF-κB p65 nuclear translocation

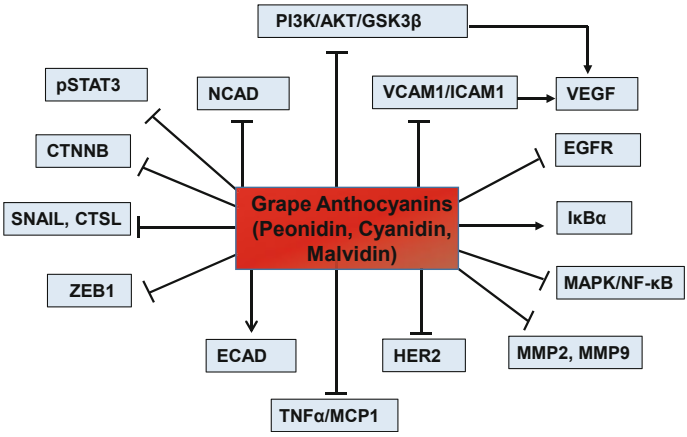

Fig. 12.7 Molecular actions of grape anthocyanins against angiogenesis and metastasis

(Huang et al. 2014). All these studies suggest direct/indirect effects of anthocyanins against tumor angiogenesis and metastasis. Figure 12.7 shows the molecular actions of grape anthocyanins against angiogenesis and metastasis.

12.4.5 Whole Grape Powder Against Angiogenesis and Metastasis

A limited number of studies have assessed the effects of whole grape powder against angiogenesis and metastasis, in certain models. Liu and colleagues tested the antiangiogenic activity of four grape varieties, Concord, Niagara, Chardonnay, and Pinot noir, and demonstrated that those with the highest total phenolics and flavonoids possess the highest antiangiogenic activity (Liu et al. 2010). Recently, we used freeze-dried grape powder, which was comprised of fresh black, green, and red grapes of both seeded and seedless varieties [provided from the California Table Grape Commission (CTGC)]. Phytochemical analysis of this grape powder showed a considerable amount of total polyphenols (3260 mg/kg in gallic acid equivalents). Dietary supplementation of this grape powder was found to protect against UVB-mediated skin carcinogenesis in female SKH-1 hairless mice (Singh et al. 2019). Moreover, the grape powder was found to improve DNA damage repair, decreased cell proliferation, increased apoptosis, and modulations in several genes involved in antioxidant function, metabolism of reactive oxygen species (ROS), superoxide metabolism and oxidative stress response (Singh et al. 2019). Additionally, proteomics analysis of tumor samples of mice supplemented with grape powder identified modulation of several proteins whose interactions and cumulative actions were linked to reduced oxidative stress through increased ROS metabolism and

reduced quantity of H_2O_2 (Mintie et al. 2019). This is an important finding as ROS, H_2O_2 and other free radicals are often produced at elevated levels in tumors and known to modulate regulatory pathways involved in angiogenesis and metastasis. In fact, targeting the redox-regulated mechanisms for antiangiogenic anticancer therapy has been suggested to overcome the limitations of single-agent antiangiogenic treatments (reviewed in Tertil et al. 2010).

Earlier, Hanausek and Spears have demonstrated significant inhibition of 7,12-dimethylbenz(*a*)anthracene (DMBA)-mediated skin tumorigenesis in SENCAR (SENsitive to CARcinogenesis) mice treated with grape powder and grape-derived antioxidants. The authors found that simultaneous dietary supplementation with grape powder and topical treatment with resveratrol reduced DMBA-induced tumor burden and cyclooxygenase-2 (COX2) expression (Hanausek et al. 2011). It's important to mention here that COX2 is an enzyme associated with inflammation, angiogenesis, and tumorigenesis. Studies in primary tumors have suggested that COX2 inhibition is a potent mechanism to reduce angiogenesis. Yao et al. have demonstrated how COX2 play roles in angiogenesis of gastric cancer via modulation of VEGF, FLT1, FLK1/KDR, ANGPT1, TIE2, MMP2, and OPN (Yao et al. 2011).

Interestingly, limited clinical studies have been done, despite the promising in vitro and in vivo studies, on the anticancer activities of grape and grape constituents. Low dose resveratrol or resveratrol-containing freeze-dried grape powder treatment to patients with colon cancer was found to inhibit WNT target genes in the normal colonic mucosa, but no change in cancer tissue (Nguyen et al. 2009). The WNT signaling pathway plays an essential role in cellular proliferation, survival, apoptosis, and angiogenesis (Olsen et al. 2017). In several clinical trials, the effect of grape powder has been assessed in multiple diseases showing beneficial effects and well tolerability (reviewed in Singh et al. 2015a). In the future, more clinical trials are needed and expected to determine the beneficial effects of grape powder in cancer patients. Figure 12.8 outlines the molecular actions of grape powder against angiogenesis and metastasis.

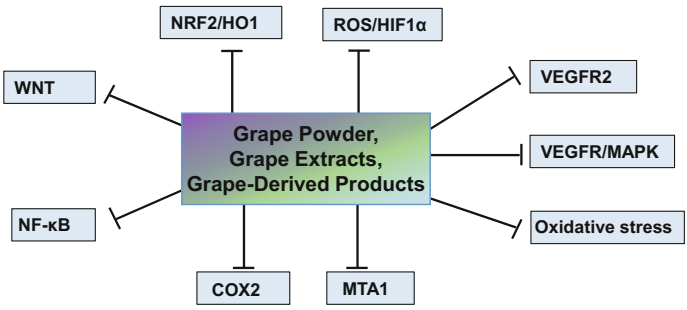

Fig. 12.8 Molecular actions of grape powder, grape extracts/grape-derived products against angiogenesis and metastasis

12.4.6 *Grape Extracts/Grape-Derived Products Against Angiogenesis and Metastasis*

Several forms of grape extracts have also been evaluated against multiple cancers. An extract of grape powder (obtained from the California Table Grape Commission) was prepared and standardized by Dr. Richard van Breemen (van Breemen et al. 2016). A photochemical analysis of this extract shows almost the same composition as of the grape powder (Singh et al. 2019; van Breemen et al. 2016). Kumar et al. (2018) have used this preparation and demonstrated the anticancer effects of grape powder extract (GPE) on cell viability, proliferation, and metastatic capability against prostate cancer cells in vitro. Upon molecular analysis, GPE, like resveratrol and pterostilbene, was found to downregulate metastasis-associated protein 1 (MTA1), which is a vital downstream target of c-MYC oncoprotein, and known to regulate EMT and metastasis (Kumar et al. 2018; Zhang et al. 2005).

Grape seed extract (GSE) has also been shown to inhibit VEGF via modulating HIF1α protein (Lu et al. 2009). Filip et al. sought to understand the protective effects of red grape seed extract against UVB-induced damages in female SKH-1 hairless mice. Topically applied GSE, before or after each UVB exposure, was found to effectively reduce cyclobutane pyrimidine dimers (CPDs), hyperplasia, cytokine release, and oxidative stress while increasing antioxidant response (Filip et al. 2011). As discussed above with grape powder, this again indicates the possibility of inhibition of angiogenesis via inhibiting oxidative stress manifested by tumor cells.

GSE has also been found to inhibit the kinase activity of purified VEGFR2, and VEGFR/MAPK-mediated signaling in endothelial cells (Wen et al. 2008). Moreover, GSE has been shown to inhibit tumor growth and angiogenesis of MDA-MB-231 tumors in mice (Wen et al. 2008). In the United States, GSE is marketed as a dietary supplement owing to their powerful protective properties against free radicals and oxidative stress.

Certain other grape-derived products, such as red wine, have also shown to affect angiogenesis. Baron-Menguy et al. have demonstrated the effects of low and high doses of red wine polyphenolic compounds (RWPC) in vivo on postischemic neovascularization in rats. Treatment with low and high doses RWPC showed pro- and anti-angiogenic properties, respectively, suggesting high dose may be beneficial against tumor angiogenesis via inhibiting VEGF expression (Baron-Menguy et al. 2007). Utilizing an uncommon part of the grape, Che et al. demonstrated that topical treatment with grape stem extracts reversed skin damage induced by the UVB radiation via decreasing lipid peroxidation, neutrophil, and mast cell infiltrations, and reducing the expressions levels of COX2, NRF2, and HO1 (Che et al. 2017). Figure 12.8 outlines the molecular actions of grape extracts/grape-derived products against angiogenesis and metastasis.

Overall, these studies suggest that grape and grape antioxidants may have strong potential towards developing complementary and alternative approaches for the inhibition of invasion/metastasis as well as angiogenesis. Therefore, further

exploration and clinical evaluation of these agents alone or in combination with the established treatment regimen is needed for the effective management of cancers.

12.5 Concluding Remarks

Cancer incidence is still on the rise, probably due to lifestyle changes and increasing longevity. Although significant advancements have been made in cancer management, there is a need for further improvement in several respects. Many harsh treatments such as radiation and chemotherapy pose severe adverse side effects and produce both short- and long-term consequences, which place further strain on the quality of life. Interestingly, grape contains a range of complex natural agents that appear to inhibit angiogenesis and metastasis by interacting with multiple pathways and by reducing tumor burden by multiple mechanisms. Considering the fact that most of the current antiangiogenesis agents, approved for clinical use or being tested in clinical trials, are associated with several adverse effects; grape or grape components as a dietary supplement appear to be an inexpensive inhibitor of angiogenesis and metastasis. Therefore, further investigations in this direction are warranted.

References

Alameddine RS, Otrock ZK, Awada A, Shamseddine A (2013) Crosstalk between HER2 signaling and angiogenesis in breast cancer: molecular basis, clinical applications and challenges. Curr Opin Oncol 25:313–324

Anborgh PH, Mutrie JC, Tuck AB, Chambers AF (2010) Role of the metastasis-promoting protein osteopontin in the tumour microenvironment. J Cell Mol Med 14:2037–2044

Ansiaux R, Baudelet C, Jordan BF, Beghein N, Sonveaux P, De Wever J et al (2005) Thalidomide radiosensitizes tumors through early changes in the tumor microenvironment. Clin Cancer Res 11:743–750

Balakrishnan S, Bhat FA, Raja Singh P, Mukherjee S, Elumalai P, Das S et al (2016) Gold nanoparticle-conjugated quercetin inhibits epithelial-mesenchymal transition, angiogenesis and invasiveness via EGFR/VEGFR-2-mediated pathway in breast cancer. Cell Prolif 49:678–697

Baron-Menguy C, Bocquet A, Guihot AL, Chappard D, Amiot MJ, Andriantsitohaina R et al (2007) Effects of red wine polyphenols on postischemic neovascularization model in rats: low doses are proangiogenic, high doses anti-angiogenic. FASEB J 21:3511–3521

Bashir R, Vale PR, Isner JM, Losordo DW (2002) Angiogenic gene therapy: pre-clinical studies and phase I clinical data. Kidney Int 61:S110–S114

Beenken A, Mohammadi M (2009) The FGF family: biology, pathophysiology and therapy. Nat Rev Drug Discov 8:235–253

Berman AY, Motechin RA, Wiesenfeld MY, Holz MK (2017) The therapeutic potential of resveratrol: a review of clinical trials. NPJ Precis Oncol 1

Bhat FA, Sharmila G, Balakrishnan S, Arunkumar R, Elumalai P, Suganya S et al (2014) Quercetin reverses EGF-induced epithelial to mesenchymal transition and invasiveness in prostate cancer (PC-3) cell line via EGFR/PI3K/Akt pathway. J Nutr Biochem 25:1132–1139

Bielenberg DR, Zetter BR (2015) The contribution of angiogenesis to the process of metastasis. Cancer J 21:267–273

Burton LJ, Smith BA, Smith BN, Loyd Q, Nagappan P, McKeithen D et al (2015) Muscadine grape skin extract can antagonize Snail-cathepsin L-mediated invasion, migration and osteoclastogenesis in prostate and breast cancer cells. Carcinogenesis 36:1019–1027

Cao Z, Fang J, Xia C, Shi X, Jiang BH (2004) Trans-3,4,5′-trihydroxystibene inhibits hypoxia-inducible factor 1alpha and vascular endothelial growth factor expression in human ovarian cancer cells. Clin Cancer Res 10:5253–5263

Carmeliet P (2003) Angiogenesis in health and disease. Nat Med 9:653–660

Carmeliet P, Jain RK (2011) Molecular mechanisms and clinical applications of angiogenesis. Nature 473:298–307

Catalgol B, Batirel S, Taga Y, Ozer NK (2012) Resveratrol: French paradox revisited. Front Pharmacol 3:141

Chan CY, Lien CH, Lee MF, Huang CY (2016) Quercetin suppresses cellular migration and invasion in human head and neck squamous cell carcinoma (HNSCC). Biomedicine (Taipei) 6:15

Chang YS, di Tomaso E, McDonald DM, Jones R, Jain RK, Munn LL (2000) Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. Proc Natl Acad Sci USA 97:14608–14613

Che DN, Xie GH, Cho BO, Shin JY, Kang HJ, Jang SI (2017) Protective effects of grape stem extract against UVB-induced damage in C57BL mice skin. J Photochem Photobiol B 173:551–559

Chen JC, Chen Y, Lin JH, Wu JM, Tseng SH (2006) Resveratrol suppresses angiogenesis in gliomas: evaluation by color Doppler ultrasound. Anticancer Res 26:1237–1245

Chen C, Zhao S, Karnad A, Freeman JW (2018a) The biology and role of CD44 in cancer progression: therapeutic implications. J Hematol Oncol 11:64

Chen X, Gu N, Xue C, Li BR (2018b) Plant flavonoid taxifolin inhibits the growth, migration and invasion of human osteosarcoma cells. Mol Med Rep 17:3239–3245

Cook NC, Samman S (1996) Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem 7:66–76

Czank C, Cassidy A, Zhang Q, Morrison DJ, Preston T, Kroon PA et al (2013) Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a (13)C-tracer study. Am J Clin Nutr 97:995–1003

Dinicola S, Pasqualato A, Cucina A, Coluccia P, Ferranti F, Canipari R et al (2014) Grape seed extract suppresses MDA-MB231 breast cancer cell migration and invasion. Eur J Nutr 53:421–431

Ebeler SE, Brenneman CA, Kim GS, Jewell WT, Webb MR, Chacon-Rodriguez L et al (2002) Dietary catechin delays tumor onset in a transgenic mouse model. Am J Clin Nutr 76:865–872

Fagiani E, Christofori G (2013) Angiopoietins in angiogenesis. Cancer Lett 328:18–26

Feng X, Ofstad W, Hawkins D (2010) Antiangiogenesis therapy: a new strategy for cancer treatment. US Pharm 35(7)(oncology suppl):4–9

Feng LL, Liu BX, Zhong JY, Sun LB, Yu HS (2014) Effect of grape procyanidins on tumor angiogenesis in liver cancer xenograft models. Asian Pac J Cancer Prev 15:737–741

Figueiras TS, Neves-Petersen MT, Petersen SB (2011) Activation energy of light induced isomerization of resveratrol. J Fluoresc 21:1897–1906

Filip A, Daicoviciu D, Clichici S, Bolfa P, Catoi C, Baldea I et al (2011) The effects of grape seeds polyphenols on SKH-1 mice skin irradiated with multiple doses of UV-B. J Photochem Photobiol B 105:133–142

Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285:1182–1186

Formica JV, Regelson W (1995) Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol 33:1061–1080

Gao Q, Yuan Y, Gan HZ, Peng Q (2015) Resveratrol inhibits the hedgehog signaling pathway and epithelial-mesenchymal transition and suppresses gastric cancer invasion and metastasis. Oncol Lett 9:2381–2387

Garvin S, Ollinger K, Dabrosin C (2006) Resveratrol induces apoptosis and inhibits angiogenesis in human breast cancer xenografts in vivo. Cancer Lett 231:113–122
Gibellini L, Pinti M, Nasi M, Montagna JP, De Biasi S, Roat E et al (2011) Quercetin and cancer chemoprevention. Evid Based Complement Alternat Med 2011:591356
Gohji K, Fidler IJ, Tsan R, Radinsky R, von Eschenbach AC, Tsuruo T et al (1994) Human recombinant interferons-beta and -gamma decrease gelatinase production and invasion by human KG-2 renal-carcinoma cells. Int J Cancer 58:380–384
Grant SW, Kyshtoobayeva AS, Kurosaki T, Jakowatz J, Fruehauf JP (1998) Mutant p53 correlates with reduced expression of thrombospondin-1, increased angiogenesis, and metastatic progression in melanoma. Cancer Detect Prev 22:185–194
Greenwell M, Rahman PK (2015) Medicinal plants: their use in anticancer treatment. Int J Pharm Sci Res 6:4103–4112
Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100:57–70
Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674
Hanausek M, Spears E, Walaszek Z, Kowalczyk MC, Kowalczyk P, Wendel C et al (2011) Inhibition of murine skin carcinogenesis by freeze-dried grape powder and other grape-derived major antioxidants. Nutr Cancer 63:28–38
Hoshino J, Park EJ, Kondratyuk TP, Marler L, Pezzuto JM, van Breemen RB et al (2010) Selective synthesis and biological evaluation of sulfate-conjugated resveratrol metabolites. J Med Chem 53:5033–5043
Hu Y, Sun CY, Huang J, Hong L, Zhang L, Chu ZB (2007) Antimyeloma effects of resveratrol through inhibition of angiogenesis. Chin Med J (Engl) 120:1672–1677
Huang CY, Chan CY, Chou IT, Lien CH, Hung HC, Lee MF (2013) Quercetin induces growth arrest through activation of FOXO1 transcription factor in EGFR-overexpressing oral cancer cells. J Nutr Biochem 24:1596–1603
Huang WY, Wang J, Liu YM, Zheng QS, Li CY (2014) Inhibitory effect of Malvidin on TNF-alpha-induced inflammatory response in endothelial cells. Eur J Pharmacol 723:67–72
Igura K, Ohta T, Kuroda Y, Kaji K (2001) Resveratrol and quercetin inhibit angiogenesis in vitro. Cancer Lett 171:11–16
Imran M, Rauf A, Shah ZA, Saeed F, Imran A, Arshad MU et al (2019) Chemo-preventive and therapeutic effect of the dietary flavonoid kaempferol: a comprehensive review. Phytother Res 33:263–275
Iqbal S, Lenz HJ (2004) Integration of novel agents in the treatment of colorectal cancer. Cancer Chemother Pharmacol 54(Suppl 1):S32–S39
Khanna S, Roy S, Bagchi D, Bagchi M, Sen CK (2001) Upregulation of oxidant-induced VEGF expression in cultured keratinocytes by a grape seed proanthocyanidin extract. Free Radic Biol Med 31:38–42
Kim SY, Ko YS, Park J, Choi Y, Park JW, Kim Y et al (2016) Forkhead transcription factor FOXO1 inhibits angiogenesis in gastric cancer in relation to SIRT1. Cancer Res Treat 48:345–354
Kim SE, Shin SH, Lee JY, Kim CH, Chung IK, Kang HM et al (2018) Resveratrol induces mitochondrial apoptosis and inhibits epithelial-mesenchymal transition in oral squamous cell carcinoma cells. Nutr Cancer 70:125–135
Kumar R, Deep G, Wempe MF, Agarwal R, Agarwal C (2015) Procyanidin B2 3,3″-di-O-gallate inhibits endothelial cells growth and motility by targeting VEGFR2 and integrin signaling pathways. Curr Cancer Drug Targets 15:14–26
Kumar A, D'Silva M, Dholakia K, Levenson AS (2018) In vitro anticancer properties of table grape powder extract (GPE) in prostate cancer. Nutrients 10:1804
Labbé D, Provençal M, Lamy S, Boivin D, Gingras D, Béliveau R (2009) The flavonols quercetin, kaempferol, and myricetin inhibit hepatocyte growth factor-induced medulloblastoma cell migration. J Nutr 139:646–652
Langley RR, Fidler IJ (2011) The seed and soil hypothesis revisited—the role of tumor-stroma interactions in metastasis to different organs. Int J Cancer 128:2527–2535

Lee J, Kim JH (2016) Kaempferol inhibits pancreatic cancer cell growth and migration through the blockade of EGFR-related pathway in vitro. PLoS One 11:e0155264

Lee SB, Cha KH, Selenge D, Solongo A, Nho CW (2007) The chemopreventive effect of taxifolin is exerted through ARE-dependent gene regulation. Biol Pharm Bull 30:1074–1079

Lee J, Han SI, Yun JH, Kim JH (2015a) Quercetin 3-O-glucoside suppresses epidermal growth factor-induced migration by inhibiting EGFR signaling in pancreatic cancer cells. Tumour Biol 36:9385–9393

Lee SH, Jeong D, Han YS, Baek MJ (2015b) Pivotal role of vascular endothelial growth factor pathway in tumor angiogenesis. Ann Surg Treat Res 89:1–8

Li C, Yang X, Chen C, Cai S, Hu J (2014) Isorhamnetin suppresses colon cancer cell growth through the PI3KAktmTOR pathway. Mol Med Rep 9:935–940

Li CL, Yang D, Zhao YW, Qiu Y, Cao X, Yu YY et al (2015) Inhibitory effects of isorhamnetin on the invasion of human breast carcinoma cells by downregulating the expression and activity of matrix metalloproteinase-2/9. Nutr Cancer 67:1191–1200

Liu M, Liu RH, Song BB, Li CF, Lin LQ, Zhang CP et al (2010) Antiangiogenetic effects of 4 varieties of grapes in vitro. J Food Sci 75:T99–T104

Liu W, Xu J, Wu S, Liu Y, Yu X, Chen J et al (2013) Selective anti-proliferation of HER2-positive breast cancer cells by anthocyanins identified by high-throughput screening. PLoS One 8: e81586

Lu J, Zhang K, Chen S, Wen W (2009) Grape seed extract inhibits VEGF expression via reducing HIF-1alpha protein expression. Carcinogenesis 30:636–644

Lu JN, Lee WS, Yun JW, Kim MJ, Kim HJ, Kim DC et al (2013) Anthocyanins from *Vitis coignetiae* Pulliat inhibit cancer invasion and epithelial-mesenchymal transition, but these effects can be attenuated by tumor necrosis factor in human uterine cervical cancer HeLa cells. Evid Based Complement Alternat Med 2013:503043

Lu JN, Lee WS, Kim MJ, Yun JW, Jung JH, Yi SM et al (2014) The inhibitory effect of anthocyanins on Akt on invasion and epithelial-mesenchymal transition is not associated with the anti-EGFR effect of the anthocyanins. Int J Oncol 44:1756–1766

Lu JN, Panchanathan R, Lee WS, Kim HJ, Kim DH, Choi YH et al (2017) Anthocyanins from the fruit of *Vitis coignetiae* Pulliat inhibit TNF-augmented cancer proliferation, migration, and invasion in A549 cells. Asian Pac J Cancer Prev 18:2919–2923

Luan YY, Liu ZM, Zhong JY, Yao RY, Yu HS (2015) Effect of grape seed proanthocyanidins on tumor vasculogenic mimicry in human triple-negative breast cancer cells. Asian Pac J Cancer Prev 16:531–535

Luo HT, Jiang BH, King SM, Chen YC (2008) Inhibition of cell growth and VEGF expression in ovarian cancer cells by flavonoids. Nutr Cancer 60:800–809

Luo H, Rankin GO, Liu L, Daddysman MK, Jiang BH, Chen YC (2009) Kaempferol inhibits angiogenesis and VEGF expression through both HIF dependent and independent pathways in human ovarian cancer cells. Nutr Cancer 61:554–563

Luo H, Rankin GO, Juliano N, Jiang BH, Chen YC (2012) Kaempferol inhibits VEGF expression and in vitro angiogenesis through a novel ERK-NFkappaB-cMyc-p21 pathway. Food Chem 130:321–328

Ma ZS, Huynh TH, Ng CP, Do PT, Nguyen TH, Huynh H (2004) Reduction of CWR22 prostate tumor xenograft growth by combined tamoxifen-quercetin treatment is associated with inhibition of angiogenesis and cellular proliferation. Int J Oncol 24:1297–1304

Maurya AK, Vinayak M (2017) Quercetin attenuates cell survival, inflammation, and angiogenesis via modulation of AKT signaling in murine T-cell lymphoma. Nutr Cancer 69:470–480

Mintie CA, Singh CK, Ndiaye MA, Barrett-Wilt GA, Ahmad N (2019) Identification of molecular targets of dietary grape-mediated chemoprevention of ultraviolet B skin carcinogenesis: a comparative quantitative proteomics analysis. J Proteome Res 18(10):3741–3751

Mukherjee S, Dudley JI, Das DK (2010) Dose-dependency of resveratrol in providing health benefits. Dose Response 8:478–500

Nguyen AV, Martinez M, Stamos MJ, Moyer MP, Planutis K, Hope C et al (2009) Results of a phase I pilot clinical trial examining the effect of plant-derived resveratrol and grape powder on Wnt pathway target gene expression in colonic mucosa and colon cancer. Cancer Manag Res 1:25–37

Oliveira IC, Sciavolino PJ, Lee TH, Vilcek J (1992) Downregulation of interleukin 8 gene expression in human fibroblasts: unique mechanism of transcriptional inhibition by interferon. Proc Natl Acad Sci U S A 89:9049–9053

Olsen JJ, Pohl SO, Deshmukh A, Visweswaran M, Ward NC, Arfuso F et al (2017) The role of Wnt signalling in angiogenesis. Clin Biochem Rev 38:131–142

Park SY, Jeong KJ, Lee J, Yoon DS, Choi WS, Kim YK et al (2007) Hypoxia enhances LPA-induced HIF-1alpha and VEGF expression: their inhibition by resveratrol. Cancer Lett 258:63–69

Patel KR, Andreadi C, Britton RG, Horner-Glister E, Karmokar A, Sale S et al (2013) Sulfate metabolites provide an intracellular pool for resveratrol generation and induce autophagy with senescence. Sci Transl Med 5:205ra133

Paul B, Masih I, Deopujari J, Charpentier C (1999) Occurrence of resveratrol and pterostilbene in age-old darakchasava, an ayurvedic medicine from India. J Ethnopharmacol 68:71–76

Payen VL, Porporato PE, Danhier P, Vazeille T, Blackman M, May BH et al (2017) (+)-Catechin in a 1:2 complex with lysine inhibits cancer cell migration and metastatic take in mice. Front Pharmacol 8:869

Pezzuto JM (2008) Grapes and human health: a perspective. J Agric Food Chem 56:6777–6784

Phromnoi K, Yodkeeree S, Anuchapreeda S, Limtrakul P (2009) Inhibition of MMP-3 activity and invasion of the MDA-MB-231 human invasive breast carcinoma cell line by bioflavonoids. Acta Pharmacol Sin 30:1169–1176

Popat R, Plesner T, Davies F, Cook G, Cook M, Elliott P et al (2013) A phase 2 study of SRT501 (resveratrol) with bortezomib for patients with relapsed and or refractory multiple myeloma. Br J Haematol 160:714–717

Pratheeshkumar P, Budhraja A, Son YO, Wang X, Zhang Z, Ding S et al (2012) Quercetin inhibits angiogenesis mediated human prostate tumor growth by targeting VEGFR-2 regulated AKT/mTOR/P70S6K signaling pathways. PLoS One 7:e47516

Pratheeshkumar P, Son YO, Wang X, Divya SP, Joseph B, Hitron JA et al (2014) Cyanidin-3-glucoside inhibits UVB-induced oxidative damage and inflammation by regulating MAP kinase and NF-kappaB signaling pathways in SKH-1 hairless mice skin. Toxicol Appl Pharmacol 280:127–137

Renaud S, de Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339:1523–1526

Risau W (1997) Mechanisms of angiogenesis. Nature 386:671–674

Sagar SM, Yance D, Wong RK (2006) Natural health products that inhibit angiogenesis: a potential source for investigational new agents to treat cancer-Part 1. Curr Oncol 13:14–26

Samant RS, Shevde LA (2011) Recent advances in anti-angiogenic therapy of cancer. Oncotarget 2:122–134

Schindler R, Mentlein R (2006) Flavonoids and vitamin E reduce the release of the angiogenic peptide vascular endothelial growth factor from human tumor cells. J Nutr 136:1477–1482

Shankar S, Nall D, Tang SN, Meeker D, Passarini J, Sharma J et al (2011) Resveratrol inhibits pancreatic cancer stem cell characteristics in human and KrasG12D transgenic mice by inhibiting pluripotency maintaining factors and epithelial-mesenchymal transition. PLoS One 6:e16530

Sheth S, Jajoo S, Kaur T, Mukherjea D, Sheehan K, Rybak LP et al (2012) Resveratrol reduces prostate cancer growth and metastasis by inhibiting the Akt/MicroRNA-21 pathway. PLoS One 7:e51655

Shi J, Yu J, Pohorly JE, Kakuda Y (2003) Polyphenolics in grape seeds-biochemistry and functionality. J Med Food 6:291–299

Singh RK, Gutman M, Bucana CD, Sanchez R, Llansa N, Fidler IJ (1995) Interferons alpha and beta down-regulate the expression of basic fibroblast growth factor in human carcinomas. Proc Natl Acad Sci U S A 92:4562–4566

Singh CK, Liu X, Ahmad N (2015a) Resveratrol, in its natural combination in whole grape, for health promotion and disease management. Ann N Y Acad Sci 1348:150–160

Singh CK, Ndiaye MA, Ahmad N (2015b) Resveratrol and cancer: challenges for clinical translation. Biochim Biophys Acta 1852:1178–1185

Singh CK, Siddiqui IA, El-Abd S, Mukhtar H, Ahmad N (2016) Combination chemoprevention with grape antioxidants. Mol Nutr Food Res 60:1406–1415

Singh CK, Chhabra G, Ahmad N (2018) Chapter 7: Resveratrol and cancer cell biology. In Wu JM, Hsieh T-c (eds) Resveratrol: state of the art science and health applications. Actionable targets and mechanisms of resveratrol. World Scientific, Singapore, pp 183–207

Singh CK, Mintie CA, Ndiaye MA, Chhabra G, Dakup PP, Ye T et al (2019) Chemoprotective effects of dietary grape powder on UVB radiation-mediated skin carcinogenesis in SKH-1 hairless mice. J Invest Dermatol 139:552–561

Sprouse AA, van Breemen RB (2016) Pharmacokinetic interactions between drugs and botanical dietary supplements. Drug Metab Dispos 44:162–171

Sun Q, Prasad R, Rosenthal E, Katiyar SK (2011) Grape seed proanthocyanidins inhibit the invasive potential of head and neck cutaneous squamous cell carcinoma cells by targeting EGFR expression and epithelial-to-mesenchymal transition. BMC Complement Altern Med 11:134

Tertil M, Jozkowicz A, Dulak J (2010) Oxidative stress in tumor angiogenesis—therapeutic targets. Curr Pharm Des 16:3877–3894

Thiery JP, Acloque H, Huang RY, Nieto MA (2009) Epithelial-mesenchymal transitions in development and disease. Cell 139:871–890

Thurston G, Daly C (2012) The complex role of angiopoietin-2 in the angiopoietin-tie signaling pathway. Cold Spring Harb Perspect Med 2:a006550

Trapp V, Parmakhtiar B, Papazian V, Willmott L, Fruehauf JP (2010) Anti-angiogenic effects of resveratrol mediated by decreased VEGF and increased TSP1 expression in melanoma-endothelial cell co-culture. Angiogenesis 13:305–315

Tseng SH, Lin SM, Chen JC, Su YH, Huang HY, Chen CK et al (2004) Resveratrol suppresses the angiogenesis and tumor growth of gliomas in rats. Clin Cancer Res 10:2190–2202

Ucuzian AA, Gassman AA, East AT, Greisler HP (2010) Molecular mediators of angiogenesis. J Burn Care Res 31:158–175

van Breemen RB, Wright B, Li Y, Nosal D (2016) Burton T standardized grape powder for basic and clinical research. In: Pezzuto JM (ed) Grapes and health. Springer, Cham, pp 17–26

van Zijl F, Krupitza G, Mikulits W (2011) Initial steps of metastasis: cell invasion and endothelial transmigration. Mutat Res 728:23–34

Walle T, Hsieh F, DeLegge MH, Oatis JE Jr, Walle UK (2004) High absorption but very low bioavailability of oral resveratrol in humans. Drug Metab Dispos 32:1377–1382

Wang H, Zhang H, Tang L, Chen H, Wu C, Zhao M et al (2013) Resveratrol inhibits TGF-beta1-induced epithelial-to-mesenchymal transition and suppresses lung cancer invasion and metastasis. Toxicology 303:139–146

Ward AB, Mir H, Kapur N, Gales DN, Carriere PP, Singh S (2018) Quercetin inhibits prostate cancer by attenuating cell survival and inhibiting anti-apoptotic pathways. World J Surg Oncol 16:108

Weber GF (2008) Molecular mechanisms of metastasis. Cancer Lett 270:181–190

Weber GF, Bronson RT, Ilagan J, Cantor H, Schmits R, Mak TW (2002) Absence of the CD44 gene prevents sarcoma metastasis. Cancer Res 62:2281–2286

Wen W, Lu J, Zhang K, Chen S (2008) Grape seed extract inhibits angiogenesis via suppression of the vascular endothelial growth factor receptor signaling pathway. Cancer Prev Res (Phila) 1:554–561

Wu H, Liang X, Fang Y, Qin X, Zhang Y, Liu J (2008) Resveratrol inhibits hypoxia-induced metastasis potential enhancement by restricting hypoxia-induced factor-1 alpha expression in colon carcinoma cells. Biomed Pharmacother 62:613–621

Yao L, Liu F, Hong L, Sun L, Liang S, Wu K et al (2011) The function and mechanism of COX-2 in angiogenesis of gastric cancer cells. J Exp Clin Cancer Res 30:13

Yilmaz M, Christofori G (2010) Mechanisms of motility in metastasizing cells. Mol Cancer Res 8:629–642

Yu HB, Zhang HF, Zhang X, Li DY, Xue HZ, Pan CE et al (2010) Resveratrol inhibits VEGF expression of human hepatocellular carcinoma cells through a NF-kappa B-mediated mechanism. Hepatogastroenterology 57:1241–1246

Zhang XY, DeSalle LM, Patel JH, Capobianco AJ, Yu D, Thomas-Tikhonenko A et al (2005) Metastasis-associated protein 1 (MTA1) is an essential downstream effector of the c-MYC oncoprotein. Proc Natl Acad Sci U S A 102:13968–13973

Zhao HC, Qin R, Chen XX, Sheng X, Wu JF, Wang DB et al (2006) Microvessel density is a prognostic marker of human gastric cancer. World J Gastroenterol 12:7598–7603

Zheng Q, Tong M, Ou BQ, Liu CZ, Hu CP, Yang Y (2019) Isorhamnetin protects against bleomycin-induced pulmonary fibrosis by inhibiting endoplasmic reticulum stress and epithelial-mesenchymal transition. Int J Mol Med 43:117–126

Zhou W, Liu ZM, Wang M, Chen DY, Zhou L, Guo L (2019) Taxifolin inhibits the development of scar cell carcinoma by inducing apoptosis, cell cycle arrest, and suppression of PI3K/AKT/mTOR pathway. J Buon 24:853–858

Zhu YC, Sun LN, Zhang HJ, Li Y, Lai ST (2017) Effects of isorhamnetin on protein expression of VEGF, MMP-2 and endostatin in Lewis lung cancer mouse. Int J Clin Exp Med 10:11488–11495