Arch. Pharm. Res. (2019) 42:1–13
https://doi.org/10.1007/s12272-018-01102-z

Online ISSN 1976-3786
Print ISSN 0253-6269

Archives of Pharmacal Research
www.springer.com/12272

REVIEW

Tumor endothelial cells as a potential target of metronomic chemotherapy

Ji Yoon Kim[1] · Young-Myeong Kim[2]

Received: 31 October 2018 / Accepted: 17 December 2018 / Published online: 2 January 2019

Abstract Drug resistance and toxic side effects are major therapeutic hurdles affecting cancer patients receiving conventional chemotherapy based on the maximum tolerated dose. Metronomic chemotherapy (MCT), a new therapeutic approach developed to avoid these problems generally, consists of the continuous administration of low-dose cytotoxic agents without extended intervals. This therapy targets the tumor microenvironment, rather than exerting a direct effect on tumor cells. As a result, the MCT regimen functionally impairs tumor endothelial cells and circulating endothelial progenitor cells, leading to tumor dormancy via anti-angiogenesis. Over the past 10 years, several studies have highlighted the impact of MCT on the tumor microenvironment and angiogenesis and demonstrated its potential as a switch from the pro-angiogenic to the anti-angiogenic state. However, the mechanisms of action are still obscure. Here, we systematically review the evidence regarding the anti-angiogenic potential of MCT as a crucial determinant of tumor dormancy and cancer treatment.

Keywords Tumor · Metronomic chemotherapy · Endothelial cells · Angiogenesis · Vessel normalization

✉ Young-Myeong Kim
ymkim@kangwon.ac.kr

[1] Department of Anesthesiology and Pain Medicine, Hanyang University Hospital, Seoul 04763, South Korea

[2] Department of Molecular and Cellular Biochemistry School of Medicine, Kangwon National University School of Medicine, Chuncheon, Gangwon-do 24341, South Korea

Introduction

Conventional or traditional cytotoxic chemotherapy is based on cycles of drug administration using the maximum tolerated dose (MTD), followed by a drug-free rest period to allow the body to recover from acute cytotoxicity (Emmenegger et al. 2004). This therapeutic regimen leads to a highly responsive efficacy by directly inducing cytotoxicity against proliferating tumor cells but may cause significant and severe side effects, including immunosuppression, liver toxicity, and cardiac and nervous injury (Galluzzi et al. 2015). Antineoplastic drugs, used in conventional chemotherapy, intrinsically kill rapidly dividing tumor cells, but they can also induce cytotoxicity against proliferating hematopoietic and immune cells, thus suppressing natural and adaptive defense mechanisms. Several chemotherapeutic agents have immunosuppressive effects, mainly associated with reduction of monocyte/macrophage function, depletion of T lymphocytes, and dysfunction of natural killer cells, leading to an impairment of the anti-tumor immune surveillance (Galluzzi et al. 2015).

To minimize adverse effects and tumor immunosuppression, cytotoxic drugs are administrated at intervals of 3–4 weeks to recover from reversible toxicity and to allow hematopoiesis and restoration of impaired immune activity. Unfortunately, tumors frequently regrow during the resting or recovery period. To address these problems, the research groups of Judah Folkman, Robert Kerbel, and Douglas Hanahan (Browder et al. 2000; Klement et al. 2000; Hanahan et al. 2000) independently proposed a new modality of drug administration called metronomic chemotherapy (MCT). This therapy consists of frequent or continuous administration of low doses (1/10 ~ 1/3 of MTD) of chemotherapeutic drugs without extended rest

periods. Thus, MCT has a great potential to improve the quality of life of cancer patients considerably.

MCT indirectly exerts antagonizing effects on tumor growth and progression by inhibiting tumor vessel formation and stimulating anticancer immune response (Pasquier et al. 2010; Maiti 2014), suggesting that the metronomic regimen can be considered as a multi-targeted therapy. A number of preclinical and clinical studies have evaluated the efficacy and tolerability of MCT regimens (Gnoni et al. 2015) and reported promising results, showing low toxicity in patients with several types of cancer (Trevisani et al. 2015; Jones et al. 2017; Trevisani et al. 2018). Recently, a phase III study in patients with metastatic colorectal cancer showed increased progression-free survival in the MCT group compared to the observation group treated with conventional chemotherapy (Simkens et al. 2015; Woo and Jung 2017). Although MCT offers significant clinical advantages, the underlying therapeutic mechanism has not been elucidated. In this review, we will discuss the potential mechanisms of MCT at the cellular and molecular levels.

Conventional Chemotherapy Versus MCT

Despite its relatively good efficacy, conventional chemotherapy still has some limitations, such as severe side effects and impaired antitumor immune response (Emmenegger et al. 2004; Galluzzi et al. 2015). MCT has been developed as an alternative therapeutic strategy for targeting tumors and overcoming the unwanted effects. MCT provides many therapeutic benefits over conventional chemotherapy regarding dose and frequency of drug administration, pharmacokinetics, drug resistance, cellular and molecular target, host toxicity, tumor immunity, and patient quality of life (Table 1). The principal difference between both regimens is their therapeutic targets. Conventional chemotherapy primarily targets the rapidly dividing tumor cells, whereas metronomic therapy mainly targets the angiogenically activated endothelial cells present in tumor blood vessels.

Cell-based mechanism of MCT action

Several cytotoxic drugs do not specifically target tumor cells, but rather interfere with cell division by inhibiting proteins and enzymes involved in DNA synthesis, cell replication, and microtubule dynamics. Thus, these drugs are cytotoxic for all rapidly dividing cells, not only tumor cells, but also bone marrow, gut mucosa, and hair follicle cells (Emmenegger et al. 2004). Recent clinical studies showed that metronomic administration of cytotoxic anticancer drugs had similar effects on the tumor without causing significant side effects, probably by targeting multiple genes and cells, when compared to conventional chemotherapy (Trevisani et al. 2015; Trevisani et al. 2018). Although metronomic therapy can induce both direct and indirect effects on tumor cells to limit their proliferation, it has the strong potential to modulate the tumor microenvironment via both anti-angiogenesis by impairing endothelial cell function and enhanced antitumor immunity by activating T-cells, resulting in restriction of tumor growth and metastasis (Pasquier et al. 2010; Maiti 2014).

Tumors are highly heterogeneous and contain a subset of cancer cells termed stem-like tumor-initiating cells (Visvader and Lindeman 2008), whose phenotype is tightly regulated by cell-extrinsic factors, such as IL-6 and IL-8, produced in stromal or immune cells within tumor microenvironments (Iliopoulos et al. 2011; Korkaya et al. 2011). A recent study demonstrated that conventional chemotherapy induces glutamate-leucine-arginine (ELR)-motif-positive chemokines, such as CXCL1, CXCL2, CXCL5, and CXCL6, in carcinoma-associated fibroblasts. This resulted in a switch of the tumor phenotype to epithelial-mesenchymal transition-driven cancer stem cells,

Table 1 Comparison of conventional and metronomic chemotherapy regimens

	Conventional chemotherapy	Metronomic chemotherapy
Dose used	Maximum tolerated dose (MTD)	Optimal biological dose (1/10 ~ 1/3 of MTD)
Dosing frequency	At defined intervals, e.g., triweekly, fortnightly, or weekly	Administrated frequently or continuously without any interruptions
Pharmacokinetics	Rise and fall of plasma drug concentration	Sustained plasma drug concentration
Target	Proliferating tumor cells	Tumor endothelial cells and angiogenesis factors
Host toxicity	Acute or cumulative toxicity	Less significant toxicity
Tumor immunity	Impairs antitumor immune response	Stimulates antitumor immune system
Intent of treatment	Effective against primary tumor (cancer eradication)	Palliative care for advanced cancer (cancer control)
Quality of life	Diminishment	Improvement

which promote their invasive behavior. However, this phenotype modulation was mostly avoided in MCT regimens that downregulate these chemokines (Chan et al. 2016). These chemokines have also angiogenic effects, which suggests that MCT can modulate the tumor microenvironment through negative regulation of tumor angiogenesis by attenuating chemokine production. Thus, MCT improves the therapeutic outcome of tumors by modulating cell-extrinsic factors derived from stromal cells within their microenvironment.

Accumulating evidence has shown that MCT strongly inhibits endothelial cell function and tumor angiogenesis compared with conventional therapy, resulting in the tumor microenvironment remodeling, which is closely connected to modulation of antitumor immunity, stromal pro-oncogenic activity, and tumor dormancy (Maiti 2014). Therefore, metronomic therapy is thought to effectively target activated endothelial cells and negatively regulate the cooperative crosstalk between tumor angiogenesis and the tumor microenvironment, leading to a high antitumor efficacy and low cytotoxicity as compared to conventional chemotherapy.

Initial evidence for anti-angiogenesis

Solid tumor growth occurs in two distinct stages, the avascular growth phase and the vascular growth phase. During the former, tumors can grow to a size of approximately 1–2 mm^3 or a few mm in diameter before their metabolic demands are restricted by the diffusion limit of oxygen and nutrients. To overcome this limitation and continue growing, tumors switch to an angiogenic phenotype (vascular phase) attracting new blood vessels from the preexisting vascular network of the surrounding healthy tissue (Folkman 2007). During the transition phase, the hypoxic tumor microenvironment secretes diffusible pro-angiogenic factors into the surrounding tissue.

Of the prominent angiogenic activators, vascular endothelial growth factor (VEGF) is the strongest inducer of tumor angiogenesis and neovascular networks that supply tumor cells with nutrients and oxygen, resulting in tumor growth, invasion, and metastasis (Folkman 2007). Tumor angiogenesis generally consists of sequential steps of activation, proliferation, and migration of endothelial cells present in the normal vasculature surrounding the tumor. This process is known as sprouting tumor angiogenesis. Based on this concept, anti-angiogenic treatments that target the VEGF/VEGF receptor (VEGFR) pathway potentially inhibit tumor vessel growth, thus abrogating tumor progression and metastasis. Currently, many humanized monoclonal antibodies (e.g., bevacizumab and ramucirumab) and small molecules (e.g., sunitinib and axitinib) that inhibit tumor angiogenesis by targeting tumor-associated endothelial cells are used in patients with advanced colorectal, lung, ovarian, and kidney cancers (Zhang et al. 2009; Roviello et al. 2017).

By studying a mouse model of tumor growth, the laboratories of Folkman and Kerbel found evidence that MCT inhibits angiogenesis as part of its antineoplastic activity without causing severe cytotoxicity (Browder et al. 2000; Klement et al. 2000). These results suggest that MCT inhibits endothelial cell function and tumor angiogenesis, limiting tumor growth and progression; they do not exclude the possibility that MCT can directly target dividing tumor cells. To confirm which cell types were mainly targeted by MCT, a comparative study was performed in a mouse model with cyclophosphamide-resistant tumors (Browder et al. 2000). When the mice were exposed to conventional cyclophosphamide treatment, they died due to accelerated tumor growth. However, metronomic cyclophosphamide therapy induced endothelial cell dysfunction and apoptosis, followed by tumor cell apoptosis and tumor growth restriction, indicating that MCT primarily targets endothelial cells. This conclusion was subsequently confirmed using different chemotherapy agents, including vinblastine, doxorubicin, and paclitaxel (Klement et al. 2000; Bocci et al. 2002).

Accumulating evidence has demonstrated that MCT effectively inhibits tumor progression and metastasis by inhibiting tumor angiogenesis, modulating the tumor microenvironment, and functionally impairing tumor endothelial cells (TECs) (Matsuda et al. 2010a; Hida et al. 2013a). The principal mode of action of MCT is the alteration of the tumor microenvironment via suppression of tumor angiogenesis, particularly by targeting TECs, which are more sensitive to metronomic administration of cytotoxic drugs than tumor cells, eventually resulting in restriction of tumor growth (Gasparini 2001). Indeed, several lines of evidence have shown that TECs are more sensitive to anticancer drugs than tumor cells, explaining why MCT is more effective in killing or inactivating TECs than tumor cells (Upreti et al. 2013).

MCT offers several benefits, such as low cytotoxicity, continuous inhibition of tumor angiogenesis, and modulation of tumor environment, consequentially resulting in tumor dormancy and disease remission, while the conventional regimen has some limitations, such as systemic toxicity, recurrence of tumor growth, and angiogenesis during the drug-free periods (Fig. 1). Although MCT is known to target highly angiogenic TECs and consequently inhibit their angiogenic activity in the growing tumor blood vessels, the exact mechanisms by which MCT inhibits angiogenic activity of TECs rather than normal endothelial cells (NECs) remains unclear. This is likely due to the phenotypic or characteristic differences between TECs and NECs (St Croix et al. 2000; Hida et al. 2013b). Thus, we

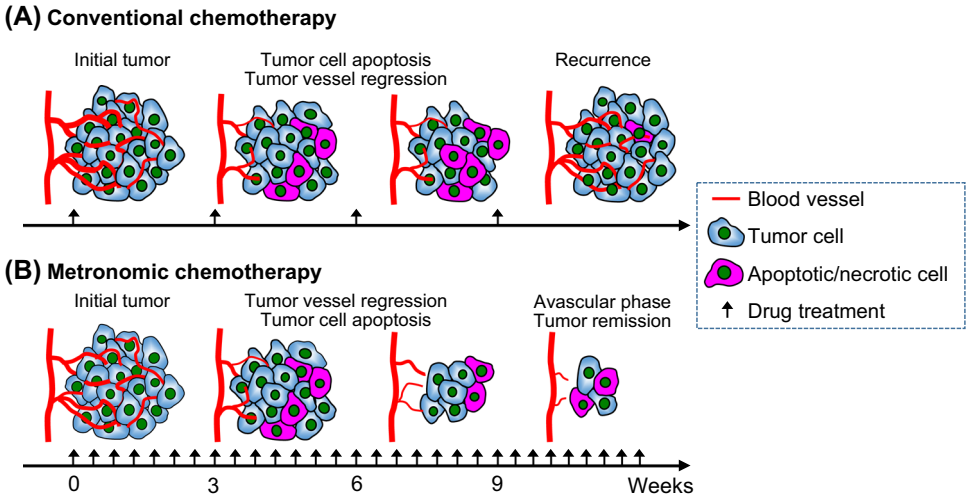

Fig. 1 Schematic representation comparing the effects of conventional and metronomic chemotherapy on tumors. **a** Conventional chemotherapeutic regimen is administrated intermittently with cytotoxic anticancer drugs at MTD, followed by a long break (e.g., 3 weeks), leading to tumor recurrence and angiogenesis after several weeks of treatment. **b** MCT is administrated frequently or continuously (e.g., every 4 days or daily), using fractionated MTD (1/10 ~ 1/3) of chemotherapeutic drugs for a period with no prolonged drug-free interruptions, resulting in effective inhibition of tumor angiogenesis, tumor growth, and consequent remission of the disease

further discuss the characteristics of TECs as a possible target for MCT.

TEC heterogeneity may allow selective inhibition

Endothelial cells form a one-cell-thick layer called endothelium, which lines the entire circulatory system, including arteries, arterioles, venules, veins, and capillaries. These cells are essential for maintaining vascular tone homeostasis, angiogenesis, and vascular integrity, which are involved in the regulation of tumor microenvironment. The microenvironment is controlled by cross-interactions occurring among various cell types, oxygen concentrations, soluble and growth factors, and extracellular matrix components, which orchestrate the fate of TECs and tumor vessel formation (Catalano et al. 2013). Among these factors, the hypoxic tumor microenvironment is a major contributor to the stabilization of the transcription factor hypoxia-inducible factor (HIF)-1α that induces the expression of various pro-angiogenic factors, including VEGF, thus promoting endothelial cell activation and tumor angiogenesis. Therefore, there is reciprocal crosstalk between tumor angiogenesis and the tumor microenvironment, leading to accelerated stimulation of TEC function and behavior. These findings suggest that TECs are a crucial and specific target for MCT to induce anti-angiogenesis and tumor dormancy.

Since TECs originate from endothelial cells existing in the normal vasculature, they have been thought to be genetically stable and identical to NECs. However, several reports have shown molecular differences between TECs and NECs, indicating that TECs are heterogeneous and not clonal at the gene expression level (St Croix et al. 2000; Hida et al. 2013b). In addition, in vitro cultured endothelial cells isolated from tumor tissue express higher levels of VEGFR-1/-2 than NECs, leading to a strong angiogenic response to VEGF (Matsuda et al. 2010b). TECs also exhibit more angiogenic properties in response to other pro-angiogenic factors, such as epidermal growth factor (EGF) and adrenomedullin, compared with NECs (Hida et al. 2013b). Notably, TECs isolated from high metastatic tumors are more angiogenic than those isolated from low metastatic tumors because they upregulate the angiogenesis-associated genes VEGFR-1/-2 and VEGF (Hida et al. 2013b), suggesting that TEC properties are closely linked to tumor malignancy. These findings indicate that TECs have remarkable heterogeneity, which may be induced by their environmental properties, particularly tumor malignancy, and that they elicit higher responses to angiogenic factors than NECs. Thus, TECs are thought to be more vulnerable to MCT than NECs (Klement et al. 2000; Bocci et al. 2002).

Because TECs have different characteristics, such as increased DNA synthesis, cell division, and proliferation, from quiescent endothelial cells in normal tissues, they are more susceptible to low-dose chemotherapeutic drugs (Kerbel and Kamen 2004). Several studies have reported that low concentrations of anticancer drugs significantly inhibit endothelial cell growth, but not tumor cell proliferation in cell culture conditions (Clements et al. 1999; Bocci et al. 2002; Klement et al. 2000). Notably, treatment with nanomolar or picomolar concentrations of vinblastine induced endothelial cell dysfunction and anti-angiogenesis without inducing apoptosis, but did not affect proliferation

of tumor cells and fibroblasts (Vacca et al. 1999), providing the possibility that MCT negatively regulates the intracellular signaling pathways of angiogenesis, without causing endothelial cytotoxicity. In addition to high sensitivity to metronomic anticancer drugs, TECs differ from NECs in other aspects, such as dividing and proliferating rate (Kerbel and Kamen 2004), angiogenic properties (Matsuda et al. 2010b), gene expression profiles (St Croix et al. 2000), epigenetic regulation (Maishi et al. 2016), and responses to growth factors (Matsuda et al. 2010a; Matsuda et al. 2011). These findings suggest that TECs display phenotypic and genotypic heterogeneities, which are considered as crucial factors for their selective sensitivity to MCT using cytotoxic drugs, despite these drugs being designed to preferentially kill the dividing and proliferating tumor cells (Kerbel 2007).

Normalization of tumor blood vessels by targeting TECs

TECs have a specialized phenotype, similar to organ-specific endothelial cells that display a significant degree of functional heterogeneity depending on their location in the different types of blood vessels, such as vascular tone in arteries, leukocyte trafficking in venules, and organ-specific properties in capillaries (Aird 2006). This heterogeneity is mostly associated with differential gene expression profiles in organ-specific and microvascular-bed-specific endothelial cells (Langenkamp and Molema 2009). This suggests that endothelial cells exhibit different behaviors in response to environmental cues in an origin-specific manner. For example, liver sinusoidal endothelial cells and kidney glomerular endothelial cells are flattened and highly fenestrated to filter or exchange fluids, solutes, and particles between plasma and the hepatocytes or the urinary space, whereas the blood–brain barrier endothelium is characterized by junctional proteins that restrict passage solutes into the central nervous system. In addition, human umbilical vein endothelial cells (HUVECs) exert dramatic differences in tumor necrosis factor (TNF)-α-induced E-selectin expression from human umbilical arterial endothelial cells, as confirmed by substantial responsiveness only in HUVECs (Liu et al. 2008). This indicates that endothelial cells are highly specialized from organ to organ, suggesting that TECs have distinct properties from endothelial cells in normal tissues.

Tumors are considered to be dysfunctional organs, because they conduct abnormal metabolic pathways, such as impaired glucose metabolism (anaerobic glucose catabolism) and increased lactate production causing acidosis (Lee et al. 2015), as well as expanded non-perfused or focal hypoxic areas, resulting in promotion of pro-angiogenic VEGF expression (Folkman 2007). Endothelial cells exposed to those environments switch to specialized phenotypes, such as TECs, which possess abnormal pro-angiogenic function (Dudley 2012). Activated TECs, present in tumor vessels, branch and sprout excessively, thus losing their polarity, detaching from the basement membrane, forming defective endothelial monolayers, and losing endothelial cell junctions (Baluk et al. 2005). As a result, tumor vessels have structural and functional abnormalities (Carmeliet and Jain 2011), such as leakiness, tortuosity, serpentine course, irregular branches, and abnormal shunts (Fig. 2). Consequently, the tumor blood flow is chaotic, with high flow rates in some segments and stagnant in others. Increased interstitial fluid pressure by focal leakage in tumor vessels further impedes tumor blood flow, leading to obstructions of chemotherapeutic drug delivery and reduction of therapeutic efficacy.

TECs activated by VEGF form the tumor microvasculature, which is generally more hyperpermeable to macromolecules than normal vessels. An early study showed that administration of anti-VEGF antibody reduced not only tumor vessel formation, but also vascular permeability in a tumor-bearing animal model (Yuan et al. 1996), suggesting that inhibition of TEC function normalizes tumor vessels and improves delivery and efficacy of chemotherapeutic drugs. Thereafter, several studies demonstrated that combined treatment of anti-angiogenic therapeutics and cytotoxic drugs synergistically inhibited tumor progression and improved survival in tumor-bearing animal models and clinical trial patients (Sandler et al. 2006; Baek et al. 2017). This indicates that MCT improves blood flow and perfusion via functional normalization of tumor vessels, leading to effective delivery of anticancer drugs into the tumor. Thus, tumor vessel normalization is currently considered as an emerging concept for metronomic anticancer drug-based tumor therapy.

In addition to an anti-angiogenic effect, MCT regimens improve structural and functional normalization of the tumor vasculature by inhibiting the expression of multiple pro-angiogenic genes, including VEGF (Colleoni et al. 2006; Cham et al. 2010). Metronomic paclitaxel therapy resulted in a significant decrease in tumor vessel permeability, an index of vascular normalization, followed by a further improvement in the antitumor effect of concomitantly injected doxorubicin in a breast cancer xenograft mouse model (Luan et al. 2016). This suggests that MCT normalizes imperfect tumor blood vessels and improves chemotherapeutic drug delivery deep inside tumor tissues. A recent study using a mathematical model demonstrated that MCT improves tumor vessel normalization, which is associated with enhanced delivery of oxygen and anticancer drugs (Mpekris et al. 2017). Thus, this therapy may be more effective in cancer treatment than conventional regimen. These findings indicate that MCT results not only

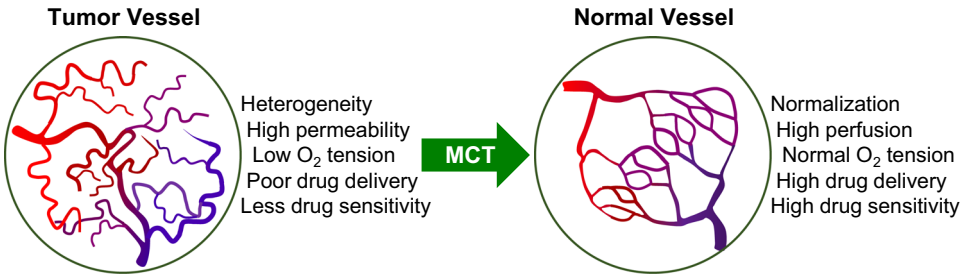

Fig. 2 Differences between normal and tumor blood vessels. Normal vasculature has hierarchical branching pattern of arteries, veins, and capillaries, whereas tumor vessels exhibit an unorganized structure, chaotic blood flow, and vascular permeability and leakiness. MCT normalizes structure and function of tumor vasculature, resulting in an improvement in drug delivery into tumor tissue and chemotherapeutic efficacy

in the inhibition of tumor angiogenesis, but also in the normalization of the tumor vasculature by effectively impairing function of TECs, but not NECs.

Functional impairment of endothelial progenitor cells (EPCs)

Postnatal new blood vessel formation, including tumor angiogenesis, has been known to result from endothelial sprouting from the preexisting vasculature. However, Asahara et al. (1997) demonstrated in 1997 that a certain population of mononuclear cells in adult peripheral blood contributed to the neovascularization of ischemic tissue by differentiating into endothelial cells. There are two types of EPCs in circulating blood, early EPCs and late EPCs (Lin et al. 2000). Early EPCs with spindle shape show peak growth at 2 to 3 weeks during in vitro culture of mononuclear cells and die at 4 weeks, whereas late EPCs with cobblestone shape appear after 2 to 3 weeks of culture show exponential growth at 4 to 8 weeks and live up to 12 weeks (Hur et al. 2004). Although both types of EPCs have comparable in vivo angiogenic capacity, early EPCs secrete more angiogenic trophic factors than late EPCs in in vitro culture conditions. Both cell types have not only therapeutic application for ischemic or hypoxic diseases but are also involved in pathological angiogenesis related to tumor growth and metastasis.

Systemic injection of EPCs into tumor-bearing mice increased vascular network formation within the tumor microenvironment and promoted tumor growth and progression (Russell and Brown 2014). This suggests that EPCs serve as a therapeutic target for inhibiting tumor growth and metastasis. Indeed, disrupting the stromal cell-derived factor-1 (SDF-1)/CXCR4 pathway, a crucial axis of EPC homing and migration, with a CXCR4-blocking antibody reduced recruitment of bone marrow-derived EPCs to the tumor bed and inhibited tumor growth in Lewis lung cancer mouse models (Murakami et al. 2009). During the recovery period after conventional chemotherapy, the number of circulating EPCs increased sharply, doubling pre-therapeutic levels at day 21 after conventional chemotherapy. However, metronomic treatment with the cyclophosphamide analog ifosfamide decreased the circulating EPC levels of cancer patients (Stoelting et al. 2008), suggesting that metronomic treatment of anticancer drugs inhibits tumor angiogenesis by decreasing circulating EPCs. MCT with irinotecan significantly also diminished EPC count in peripheral blood and tumor vessel density compared with conventional therapy in a mouse model for colon cancer (Murakami et al. 2011).

Similarly, metronomic treatment with topotecan in combination with the tyrosine kinase inhibitor pazopanib significantly reduced the number of circulating EPCs in tumor-bearing mice, leading to a parallel reduction in tumor angiogenesis and vessel density (Kumar et al. 2011). Several other studies also demonstrated that the antitumor efficacy of MCT is associated with angiogenic dormancy via the inhibitory effects on mobilization and viability of circulating EPCs in patients with several types of cancers (Stoelting et al. 2008; Natale and Bocci 2018). These findings strongly suggest that functional impairment of EPCs is a mechanism of MCT.

Molecular targets of MCT

MCT exerts its anti-angiogenic effects not only by inhibiting the angiogenic properties of TECs and EPCs but also by differentially regulating expression of pro- and anti-angiogenic genes. Angiogenic activity largely depends on local alterations of the net balance between angiogenic stimulators and inhibitors that are usually expressed in the tumor microenvironment (Fig. 3). In the following sections, we will discuss the asymmetric expression of pro- and anti-angiogenic genes, as molecular targets of MCT, in preclinical and clinical settings.

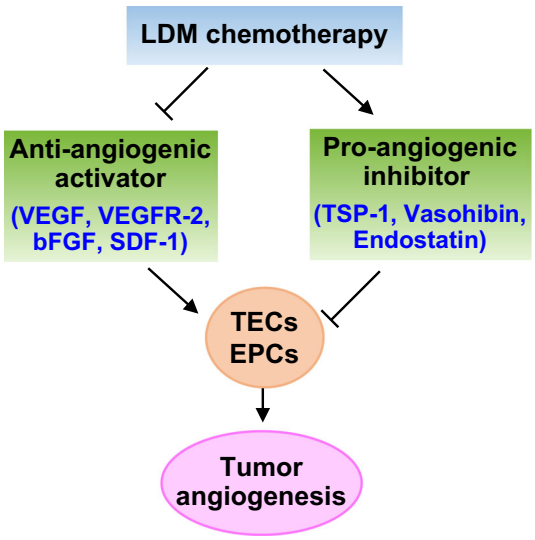

Fig. 3 Schematic representation illustrating the anti-angiogenic mechanisms of MCT. MCT inhibits the expression of pro-angiogenic factors, such as VEGF, VEGFR-2, bFGF, and SDF-1, and also increases the expression of anti-angiogenic factors, such as TSP-1, vasohibin, and endostatin. The changes in the levels of both factors inhibit angiogenic activity of TECs and suppresses mobilization and recruitment of EPCs, resulting in inhibition of angiogenesis in the tumor microenvironment

Pro-angiogenic factors

The potent angiogenic stimulator VEGF is induced and secreted from the hypoxic tumor microenvironment and diffuses to nearby vessels in normal tissues. It binds to VEGFR-2 expressed in vascular endothelial cells and triggers a molecular signal cascade of tumor angiogenesis. Therefore, the humanized anti-VEGF antibody bevacizumab is clinically used to treat various types of solid tumors (Sandler et al. 2006; Roviello et al. 2017), indicating that blocking VEGF expression is a useful strategy for cancer treatment.

Metronomic treatment of colorectal and gastric cancer cells with capecitabine decreased VEGF expression and tumor angiogenesis (Shi et al. 2014; Yuan et al. 2015), suggesting that decreased VEGF levels are directly associated with the antitumor effects of MCT. A similar decrease in serum VEGF concentration was observed in tumor-bearing mice receiving subcutaneous MCT (Mainetti et al. 2012). Furthermore, oral treatment of breast cancer patients with metronomic cyclophosphamide effectively diminished VEGF serum levels and significantly inhibited tumor growth (Colleoni et al. 2006; Bazzola et al. 2015), resulting in a significant improvement in progression-free survival (Calleri et al. 2009). These results provide evidence that MCT regimen impairs angiogenic activity of TECs through downregulation of VEGF in the tumor microenvironment.

MCT also caused a significant decrease in other pro-angiogenic factors, such as EGF, platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF). A recent study confirmed that metronomic treatment with capecitabine reduced PDGF levels in the culture supernatants of gastric cancer cells and the peripheral blood of tumor-bearing nude mice (Yuan et al. 2015). Metronomic administration of the drug GMX1777, a cellular NAD synthesis inhibitor, resulted in decreases in VEGF and PDGF expression, tumor vessel maturation, and tumor progression in a human neuroblastoma xenograft mouse model (Fuchs et al. 2010). It was also demonstrated that metronomic gemcitabine or etoposide diminished the expression levels of several pro-angiogenic molecules, such as EGF and bFGF (Cham et al. 2010; Panigrahy et al. 2010). Therefore, MCT might elicit its anti-angiogenic effects by causing a dynamic inversion of the pro-angiogenic disequilibrium through downregulation of multiple pro-angiogenic genes in the tumor microenvironment.

Anti-angiogenic factors

Many angiogenic inhibitors, including anti-VEGF antibodies and small chemical drugs, have been developed, and various endogenous inhibitors of angiogenesis have been also discovered. Both are clinically used in anti-angiogenic tumor therapy. Endogenous angiogenesis inhibitors include proteins, protein fragments, or peptides; for example, thrombospondins (TSP), pigment epithelium-derived factor (PEDF), chondromodulin-1, platelet factor-4, soluble VEGFR-1 (sVEGFR-1/sFlt-1), sVEGFR-2, soluble endoglin, vasohibins, endostatin, angiostatin, and tumstatin (Rao et al. 2015).

In addition to the decreased levels of pro-angiogenic factors, Bocci et al. showed that MCT using paclitaxel and cyclophosphamide increased TSP-1 expression in endothelial cells and its circulating levels in the plasma of human tumor-bearing mice (Bocci et al. 2003). They also demonstrated that the anti-angiogenic and antitumoral effects of metronomic cyclophosphamide were lost in TSP-1-null mice carrying Lewis lung carcinomas. Although the underlying mechanism remains unclear, MCT with different chemotherapeutic drugs significantly inhibited tumor angiogenesis and progression by increasing TSP-1 expression and production in tumor tissues of animals carrying various types of tumors, including pancreatic adenocarcinomas, colorectal cancer, hepatocellular carcinoma, ovarian cancer, breast cancer, and gastric cancer (Shi et al. 2014; Natale and Bocci 2018).

A notable increase in TSP-1 levels has also been found in cancer patients enrolled in clinical trials of various phases. Indeed, metronomic irinotecan administration enhanced plasma TSP-1 levels in metastatic colorectal

cancer patients (Allegrini et al. 2008). Similar increases in TSP-1 plasma levels were confirmed in metastatic gastrointestinal cancer patients receiving MCT with cyclophosphamide, uracil-tegafur, and celecoxib (Allegrini et al. 2012). Oral metronomic vinorelbine and dexamethasone also induced a significant increase in plasma TSP-1 levels in castration-resistant prostate cancer patients, leading to favorable therapeutic activity without significant toxicity profile (Di Desidero et al. 2016).

In addition to TSP-1, other endogenous angiogenesis inhibitors are enhanced by MCT. In fact, metronomic treatments with anticancer drugs significantly increased the plasma levels of sVEGFR-1/-2, endostatin, and PEDF, which were inversely correlated with angiogenesis or tumor progression in preclinical and clinical settings (Panigrahy et al. 2010; Jia and Waxman 2013; Natale and Bocci 2018). However, the anti-angiogenic capacity of MCT was diminished in TSP-1-deficient mice, but not in type XVIII collagen α1 chain (endostatin)- and type IV collagen α3 chain (tumstatin)-deficient mice (Hamano et al. 2004), suggesting that plasma TSP-1 level could serve as an anti-angiogenic marker for the success of MCT in cancer patients. Noticeably, MCT regimens did not always induce increased plasma levels of anti-angiogenic factors in cancer patients, nor is there any significant correlation between anti-angiogenic factor levels and clinical outcomes of patients (Gnoni et al. 2015), suggesting that efficacy of MCT is associated with multiple factors.

Factors that influence EPC activity

EPCs not only reduce ischemic damage by promoting neovascularization but also promote tumor angiogenesis, leading to tumor progression and metastasis. In general, VEGF and SDF-1 recruit EPCs from the bone marrow or activate tumor-resident EPCs. Both factors are upregulated in a HIF-1α-dependent manner (Ceradini et al. 2004), indicating that recruitment of EPCs to the tumor bed can be mediated by hypoxia-dependent expression of VEGF and SDF-1 within the tumor microenvironment. Indeed, combination of VEGF and SDF-1 resulted in a synergistic stimulation of EPC-mediated angiogenesis and blood perfusion in a murine model of hind-limb ischemia (Yu et al. 2009).

SDF-1 is a chemokine that plays a major role in mobilization, trafficking, homing, and retention of hematopoietic $CD34^+$ stem cells, including EPCs, via ligation of its sole receptor CXCR4. Disruption of the SDF-1/CXCR4 interaction by a blocking antibody against CXCR4 or SDF-1 significantly reduced tumor growth and metastasis in tumor-bearing mice, subsequently promoting their survival (Muller et al. 2001; Jung et al. 2017). This suggests that SDF-1 suppression inhibits tumor angiogenesis by impairing CXCR4-mediated homing of circulating EPCs to the tumor environment. Similarly, metronomic administration of anthracycline inhibited HIF-1α-dependent SDF-1 expression and EPC recruitment, resulting in a significant reduction of tumor vascularization in prostate cancer xenograft mice (Lee et al. 2009). These findings suggest that MCT inhibits EPC-induced tumor angiogenesis by downregulating SDF-1.

Regulated in development and DNA damage response 1 (REDD1) as an endogenous inhibitor of mTOR

Cytotoxic anticancer drugs, including DNA damage-inducing doxorubicin, significantly upregulate REDD1 gene (Räsänen et al. 2016). This gene is highly induced by various stressful conditions, such as oxidative stress, DNA damage, and energy stress (Ellisen et al. 2002). Notably, chemotherapeutic drugs induce REDD1 expression by inducing oxidative stress and p53 (Vadysirisack et al. 2011; Nissinen et al. 2016). REDD1 is an endogenous inhibitor of mTOR, which stimulates protein biosynthesis by phosphorylating ribosomal protein S6 kinase (S6K) and eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1). Doxorubicin frequently leads to heart failure and vascular dysfunction, and these events were recovered by adeno-associated viral vector-mediated gene transfer of VEGF (Räsänen et al. 2016). These findings suggest that metronomic doxorubicin may inhibit VEGF expression at the translational level by inhibiting mTOR-dependent phosphorylation of S6K and 4E-BP1 via upregulation of REDD1.

Accumulating evidence showed that mTOR stimulates physiological and pathological angiogenesis, including tumor angiogenesis, probably via translational upregulation of pro-angiogenic factors, suggesting that mTOR inhibitors prevent tumor angiogenesis by impairing endothelial cell function (Frost et al. 2013). Several studies have demonstrated that the mTOR inhibitor rapamycin blocks VEGF expression in patients with stable angina and cultured endothelial cells and that it inhibits primary and metastatic tumor growth by promoting anti-angiogenesis (Guba et al. 2002; Czepluch and Waltenberger 2008; Wang et al. 2016). Other studies have shown that rapamycin attenuates translation/stabilization of HIF-1α protein and subsequent downregulation of VEGF (Wei et al. 2016). Moreover, mice with endothelial cell-specific knockout of DEP domain-containing mTOR-interacting protein (DEPTOR), a repressor of mTOR signaling, hyperactivate the mTOR pathway and promote angiogenesis via HIF-1α-dependent VEGF expression. These angiogenic events were blocked by rapamycin (Ding et al. 2018), indicating that the mTOR pathway stimulates tumor angiogenesis by upregulating

VEGF. Similarly, specific knockdown of the mTORC2 component rapamycin-insensitive companion of mammalian target of rapamycin (RICTOR) impaired tumor growth in pancreatic cancer models by inhibiting VEGF expression and secretion (Schmidt et al. 2017). These results strongly suggest that REDD1 may mitigate tumor angiogenesis by impairing mTOR-dependent translational biosynthesis of VEGF and VEGFR-2 (Fig. 4), although it has not been proved that REDD1 directly inhibits VEGF expression.

The Sox transcription factor family is divided into several subfamilies, particularly with the SoxF subfamily consisting of Sox7, Sox17, and Sox18 (Sarkar and Hochedlinger 2013). Sox7 and Sox17 are specifically expressed in endothelial cells and stimulate sprouting angiogenesis by promoting VEGFR-2 expression; notably, Sox7 plays an important role in tumor angiogenesis (Kim et al. 2016). Expression of Sox7 and Sox17 is negatively regulated by rapamycin and positively regulated by overexpression mTOR (Kim et al. 2016), suggesting that mTOR is essential for SoxF-mediated VEGFR-2 expression and tumor angiogenesis. Specific deletion of Sox7 in endothelial cells diminishes tumor growth in mice with high-grade glioma by suppressing VEGFR-2 expression

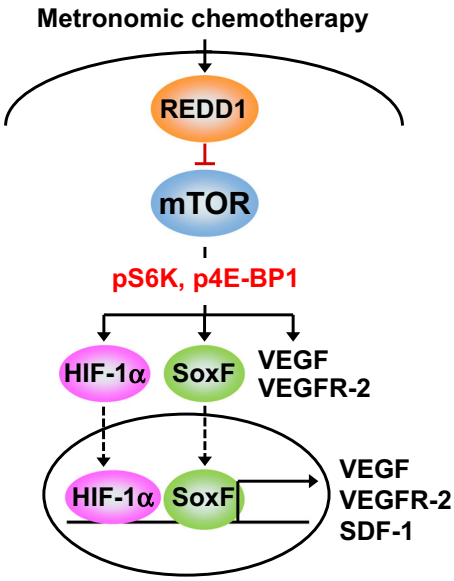

Fig. 4 Possible role of REDD1 in MCT-induced downregulation of pro-angiogenic genes. MCT induces REDD1 via DNA damage or reactive oxygen species (ROS) production. REDD1 inhibits mTOR-dependent translational systems, such as phosphorylation of ribosomal S6 protein kinase (S6K) and eukaryotic initiation factor 4E-binding protein (4E-BP1), and directly downregulates the biosynthesis of pro-angiogenic factors (VEGF and VEGFR-2) and transcription factors (HIF-1α and SoxF), resulting in transcriptional inhibition of pro-angiogenic genes, including VEGF, VEGFR-2, and SDF-1. Consequently, MCT blocks tumor angiogenesis and promotes tumor dormancy

(Kim et al. 2018). These results suggest that REDD1 induced by MCT may contribute to anti-angiogenesis by inhibiting translational biosynthesis of HIF-1α and SoxF, resulting in transcriptional downregulation of pro-angiogenic factors, such as VEGF, VEGFR-2, and SDF-1 (Fig. 4).

Translational initiation in eukaryotes occurs via two main mechanisms that are either dependent or independent on the m^7GTP cap of mRNA, namely the canonical cap-dependent and the alternative cap-independent translation (Merrick 2004). The mTOR pathway affects mRNA translation through phosphorylation of downstream targets, such as S6K and 4E-BP1, facilitating the formation of the translation initiation complex on the 5′-cap of mRNA transcripts. Rapamycin inhibits 4E-BP1 phosphorylation and cap-dependent initiation of translation (Beretta et al. 1996), suggesting that REDD1 can block cap-dependent protein biosynthesis. The antifungal agent itraconazole was shown to inhibit the mTOR signaling pathways and VEGFR-2 expression in cultured endothelial cells and to reduce tumor growth by promoting anti-angiogenesis (Choi et al. 2017). These effects were further augmented by co-treatment with paclitaxel that inhibits the mTOR pathway (Rocha et al. 2011; Choi et al. 2017). These results indicate that REDD1, induced by MCT, plays an important role in the inhibition of VEGF-mediated tumor angiogenesis.

Conclusions and future perfectives

Based on the available experimental, pre-clinical, and clinical data, it seems worthwhile to establish MCT as a new clinical treatment strategy to control several types of cancer, although the underlying mechanisms are only partially understood. Conventional chemotherapy directly targets proliferating tumor cells, whereas MCT can act on several different targets, such as the tumor microenvironment, the immune system, endothelial cells, and circulating EPCs. Among them, TECs and EPCs are the primary targets of MCT. As a result, MCT regimen effectively inhibits tumor angiogenesis. Overall, MCT provides a better quality of life with minimal toxicity and fewer complications in cancer patients. The molecular targets of MCT are still obscure; therefore, omics-based pharmacogenetic and pharmacoproteomic approaches are needed to identify precise molecular targets of TECs in response to MCT and to determine the most effective drugs and/or combinations for clinical use. It should be noted that accumulating data from phase II/III studies have shown promising outcomes in patients with several types of cancers following MCT (Simkens et al. 2015; Woo and Jung 2017; Trevisani et al. 2018). A randomized, double-blind phase III study is

required in the near future to evaluate the prospective benefits of MCT precisely.

Acknowledgements This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NRF-2017R1A2B3004565).

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.

References

Aird WC (2006) Mechanisms of endothelial cell heterogeneity in health and disease. Circ Res 98:159–162

Allegrini G, Falcone A, Fioravanti A, Barletta MT, Orlandi P, Loupakis F, Cerri E, Masi G, Di Paolo A, Kerbel RS, Danesi R, Del Tacca M, Bocci G (2008) A pharmacokinetic and pharmacodynamic study on metronomic irinotecan in metastatic colorectal cancer patients. Br J Cancer 98:1312–1319

Allegrini G, Di Desidero T, Barletta MT, Fioravanti A, Orlandi A, Canu B, Chericoni S, Loupakis F, Di Paolo A, Masi G, Fontana A, Lucchesi S, Arrighi G, Giusiani M, Ciarlo A, Brandi G, Danesi R, Kerbel RS, Falcone A, Bocci G (2012) Clinical, pharmacokinetic and pharmacodynamic evaluations of metronomic UFT and cyclophosphamide plus celecoxib in patients with advanced refractory gastrointestinal cancers. Angiogenesis 15:275–286

Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science 275:964–967

Baek YY, Lee DK, Kim J, Kim JH, Park W, Kim T, Han S, Jeoung D, You JC, Lee H, Won MH, Ha KS, Kwon YG, Kim YM (2017) Arg-Leu-Tyr-Glu tetrapeptide inhibits tumor progression by suppressing angiogenesis and vascular permeability via VEGF receptor-2 antagonism. Oncotarget 8:11763–11777

Baluk P, Hashizume H, McDonald DM (2005) Cellular abnormalities of blood vessels as targets in cancer. Curr Opin Genet Dev 15:102–111

Bazzola L, Foroni C, Andreis D, Zanoni V, Cappelletti MR, Allevi G, Aguggini S, Strina C, Milani M, Venturini S, Ferrozzi F, Giardini R, Bertoni R, Turley H, Gatter K, Petronini PG, Fox SB, Harris AL, Martinotti M, Berruti A, Bottini A, Reynolds AR, Generali D (2015) Combination of letrozole, metronomic cyclophosphamide and sorafenib is well-tolerated and shows activity in patients with primary breast cancer. Br J Canc 112:52–60

Beretta L, Gingras AC, Svitkin YV, Hall MN, Sonenberg N (1996) Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. EMBO J 15:658–664

Bocci G, Nicolaou KC, Kerbel RS (2002) Protracted low-dose effects on human endothelial cell proliferation and survival in vitro reveal a selective antiangiogenic window for various chemotherapeutic drugs. Cancer Res 62:6938–6943

Bocci G, Francia G, Man S, Lawler J, Kerbel RS (2003) Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy. Proc Natl Acad Sci USA 100:12917–12922

Browder T, Butterfield CE, Kraling BM, Shi B, Marshall B, O'Reilly MS, Folkman J (2000) Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. Cancer Res 60:1878–1886

Calleri A, Bono A, Bagnardi V, Quarna J, Mancuso P, Rabascio C, Dellapasqua S, Campagnoli E, Shaked Y, Goldhirsch A, Colleoni M, Bertolini F (2009) Predictive potential of angiogenic growth factors and circulating endothelial cells in breast cancer patients receiving metronomic chemotherapy plus bevacizumab. Clin Canc Res 15:7652–7657

Carmeliet P, Jain RK (2011) Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nat Rev Drug Discov 10:417–427

Catalano V, Turdo A, Di Franco S, Dieli F, Todaro M, Stassi G (2013) Tumor and its microenvironment: a synergistic interplay. Semin Cancer Biol 23:522–532

Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC (2004) Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 10:858–864

Cham KK, Baker JH, Takhar KS, Flexman JA, Wong MQ, Owen DA, Yung A, Kozlowski P, Reinsberg SA, Chu EM, Chang CW, Buczkowski AK, Chung SW, Scudamore CH, Minchinton AI, Yapp DT, Ng SS (2010) Metronomic gemcitabine suppresses tumour growth, improves perfusion, and reduces hypoxia in human pancreatic ductal adenocarcinoma. Br J Cancer 103:52–60

Chan TS, Hsu CC, Pai VC, Liao WY, Huang SS, Tan KT, Yen CJ, Hsu SC, Chen WY, Shan YS, Li CR, Lee MT, Jiang KY, Chu JM, Lien GS, Weaver VM, Tsai KK (2016) Metronomic chemotherapy prevents therapy-induced stromal activation and induction of tumor-initiating cells. J Exp Med 213:2967–2988

Choi CH, Ryu JY, Cho YJ, Jeon HK, Choi JJ, Ylaya K, Lee YY, Kim TJ, Chung JY, Hewitt SM, Kim BG, Bae DS, Lee JW (2017) The anti-cancer effects of itraconazole in epithelial ovarian cancer. Sci Rep 7:6552

Clements MK, Jones CB, Cumming M, Daoud SS (1999) Antiangiogenic potential of camptothecin and topotecan. Cancer Chemother Pharmacol 44:411–416

Colleoni M, Orlando L, Sanna G, Rocca A, Maisonneuve P, Peruzzotti G, Ghisini R, Sandri MT, Zorzino L, Nole F, Viale G, Goldhirsch A (2006) Metronomic low-dose oral cyclophosphamide and methotrexate plus or minus thalidomide in metastatic breast cancer: antitumor activity and biological effects. Ann Oncol 17:232–238

Czepluch FS, Waltenberger J (2008) Vascular endothelial growth factor protein levels and gene expression in peripheral monocytes after stenting: a randomized comparative study of sirolimus-eluting and bare-metal stents. Eur Heart J 29:1924–1925

Di Desidero T, Derosa L, Galli L, Orlandi P, Fontana A, Fioravanti A, Marconcini R, Giorgi M, Campi B, Saba A, Lucchesi S, Felipetto R, Danesi R, Francia G, Allegrini G, Falcone A, Bocci G (2016) Clinical, pharmacodynamic and pharmacokinetic results of a prospective phase II study on oral metronomic vinorelbine and dexamethasone in castration-resistant prostate cancer patients. Invest New Drugs 34:760–770

Ding Y, Shan L, Nai W, Lin X, Zhou L, Dong X, Wu H, Xiao M, Zhou X, Wang L, Li T, Fu Y, Lin Y, Jia C, Dai M, Bai X (2018) DEPTOR deficiency-mediated mTORc1 hyperactivation in vascular endothelial cells promotes angiogenesis. Cell Physiol Biochem 46:520–531

Dudley AC (2012) Tumor endothelial cells. Cold Spring Harb Perspect Med 2:a006536

Ellisen LW, Ramsayer KD, Johannessen CM, Yang A, Beppu H, Minda K, Oliner JD, McKeon F, Haber DA (2002) REDD1, a developmentally regulated transcriptional target of p63 and p53,

links p63 to regulation of reactive oxygen species. Mol Cell 10:995–1005
Emmenegger U, Man S, Shaked Y, Francia G, Wong JW, Hicklin DJ, Kerbel RS (2004) A comparative analysis of low-dose metronomic cyclophosphamide reveals absent or low-grade toxicity on tissues highly sensitive to the toxic effects of maximum tolerated dose regimens. Cancer Res 64:3994–4000
Folkman J (2007) Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov 6:273–286
Frost P, Berlanger E, Mysore V, Hoang B, Shi Y, Gera J, Lichtenstein A (2013) Mammalian target of rapamycin inhibitors induce tumor cell apoptosis in vivo primarily by inhibiting VEGF expression and angiogenesis. J Oncol 2013:897025
Fuchs D, Rodriguez A, Eriksson S, Christofferson R, Sundberg C, Azarbayjani F (2010) Metronomic administration of the drug GMX1777, a cellular NAD synthesis inhibitor, results in neuroblastoma regression and vessel maturation without inducing drug resistance. Int J Cancer 126:2773–2789
Galluzzi L, Buqué A, Kepp O, Zitvogel L (2015) Kroemer G (2015) Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell 28:690–714
Gasparini G (2001) Metronomic scheduling: the future of chemotherapy? Lancet Oncol 2:733–740
Gnoni A, Silvestris N, Licchetta A, Santini D, Scartozzi M, Ria R, Pisconti S, Petrelli F, Vacca A, Lorusso V (2015) Metronomic chemotherapy from rationale to clinical studies: a dream or reality? Crit Rev Oncol Hematol 95:46–61
Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, Jauch KW, Geissler EK (2002) Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med 8:128–135
Hamano Y, Sugimoto H, Soubasakos MA, Kieran M, Olsen BR, Lawler J, Sudhakar A, Kalluri R (2004) Thrombospondin-1 associated with tumor microenvironment contributes to low-dose cyclophosphamide-mediated endothelial cell apoptosis and tumor growth suppression. Cancer Res 64:1570–1574
Hanahan D, Bergers G, Bergsland E (2000) Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. J Clin Invest 105:1045–1047
Hida K, Akiyama K, Ohga N, Maishi N, Hida Y (2013a) Tumour endothelial cells acquire drug resistance in a tumour microenvironment. J Biochem 153:243–249
Hida K, Ohga N, Akiyama K, Maishi N, Hida Y (2013b) Heterogeneity of tumor endothelial cells. Cancer Sci 104:1391–1395
Hur J, Yoon CH, Kim HS, Choi JH, Kang HJ, Hwang KK, Oh BH, Lee MM, Park YB (2004) Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. Arterioscler Thromb Vasc Biol 24:288–293
Iliopoulos D, Hirsch HA, Wang G, Struhl K (2011) Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. Proc Natl Acad Sci USA 108:1397–1402
Jia L, Waxman DJ (2013) Thrombospondin-1 and pigment epithelium-derived factor enhance responsiveness of KM12 colon tumor to metronomic cyclophosphamide but have disparate effects on tumor metastasis. Canc Lett 330:241–249
Jones BS, Jerome MS, Miley D, Jackson BE, DeShazo MR, Reddy VV, Singh KP, Brown OC, Robert F (2017) Pilot phase II study of metronomic chemotherapy in combination with bevacizumab in patients with advanced non-squamous non-small cell lung cancer. Lung Cancer 106:125–130
Jung K, Heishi T, Incio J, Huang Y, Beech EY, Pinter M, Ho WW, Kawaguchi K, Rahbari NN, Chung E, Kim JK, Clark JW, Willett CG, Yun SH, Luster AD, Padera TP, Jain RK, Fukumura D (2017) Targeting CXCR4-dependent immunosuppressive Ly6C^{low} monocytes improves antiangiogenic therapy in colorectal cancer. Proc Natl Acad Sci USA 114:10455–10460
Kerbel RS (2007) Improving conventional or low dose metronomic chemotherapy with targeted antiangiogenic drugs. Cancer Res Treat 39:150–159
Kerbel RS, Kamen BA (2004) The anti-angiogenic basis of metronomic chemotherapy. Nat Rev Cancer 4:423–436
Kim K, Kim IK, Yang JM, Lee E, Koh BI, Song S, Park J, Lee S, Choi C, Kim JW, Kubota Y, Koh GY, Kim I (2016) SoxF transcription factors are positive feedback regulators of VEGF signaling. Circ Res 119:839–852
Kim IK, Kim K, Lee E, Oh DS, Park CS, Park S, Yang JM, Kim JH, Kim HS, Shima DT, Kim JH, Hong SH, Cho YH, Kim YH, Park JB, Koh GY, Ju YS, Lee HK, Lee S, Kim I (2018) Sox7 promotes high-grade glioma by increasing VEGFR2-mediated vascular abnormality. J Exp Med 215:963–983
Klement G, Baruchel S, Rak J, Man S, Clark K, Hicklin DJ, Bohlen P, Kerbel RS (2000) Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. J Clin Invest 105:R15–R24
Korkaya H, Liu S, Wicha MS (2011) Breast cancer stem cells, cytokine networks, and the tumor microenvironment. J Clin Invest 121:3804–3809
Kumar S, Mokhtari RB, Sheikh R, Wu B, Zhang L, Xu P, Man S, Oliveira ID, Yeger H, Kerbel RS, Baruchel S (2011) Metronomic oral topotecan with pazopanib is an active antiangiogenic regimen in mouse models of aggressive pediatric solid tumor. Clin Canc Res 17:5656–5667
Langenkamp E, Molema G (2009) Microvascular endothelial cell heterogeneity: general concepts and pharmacological consequences for anti-angiogenic therapy of cancer. Cell Tissue Res 335:205–222
Lee K, Qian DZ, Rey S, Wei H, Liu JO, Semenza GL (2009) Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor induced mobilization of circulating angiogenic cells. Proc Natl Acad Sci USA 106:2353–2358
Lee DC, Sohn HA, Park ZY, Oh S, Kang YK, Lee KM, Kang M, Jang YJ, Yang SJ, Hong YK, Noh H, Kim JA, Kim DJ, Bae KH, Kim DM, Chung SJ, Yoo HS, Yu DY, Park KC, Yeom YI (2015) A lactate-induced response to hypoxia. Cell 161:595–609
Lin Y, Weisdorf D, Solovey A, Hebbel RP (2000) Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest 105:71–77
Liu M, Kluger MS, D'Alessio A, Garcia-Cardena G, Pober JS (2008) Regulation of arterial-venous differences in tumor necrosis factor responsiveness of endothelial cells by anatomic context. Am J Pathol 172:1088–1099
Luan X, Guan YY, Lovell JF, Zhao M, Lu Q, Liu YR, Liu HJ, Gao YG, Dong X, Yang SC, Zheng L, Sun P, Fang C, Chen HZ (2016) Tumor priming using metronomic chemotherapy with neovasculature-targeted, nanoparticulate paclitaxel. Biomaterials 95:60–73
Mainetti LE, Rico MJ, Fernandez-Zenobi MV, Perroud HA, Roggero EA, Rozados VR, Scharovsky OG (2012) Therapeutic efficacy of metronomic chemotherapy with cyclophosphamide and doxorubicin on murine mammary adenocarcinomas. Ann Oncol 24:2310–2316
Maishi N, Ohba Y, Akiyama K, Ohga N, Hamada J, Nagao-Kitamoto H, Alam MT, Yamamoto K, Kawamoto T, Inoue N, Taketomi A, Shindoh M, Hida Y, Hida K (2016) Tumour endothelial cells in high metastatic tumours promote metastasis via epigenetic dysregulation of biglycan. Sci Rep 6:28039
Maiti R (2014) Metronomic chemotherapy. J Pharmacol Pharmacother 5:186–192

Matsuda K, Ohga N, Hida Y, Muraki C, Tsuchiya K, Kurosu T, Akino T, Shih SC, Totsuka Y, Klagsbrun M, Shindoh M, Hida K (2010a) Adrenomedullin antagonist suppresses tumor formation in renal cell carcinoma through inhibitory effects on tumor endothelial cells and endothelial progenitor mobilization. Int J Oncol 394:947–954

Matsuda K, Ohga N, Hida Y, Muraki C, Tsuchiya K, Kurosu T, Akino T, Shih SC, Totsuka Y, Klagsbrun M, Shindoh M, Hida K (2010b) Isolated tumor endothelial cells maintain specific character during long-term culture. Biochem Biophys Res Commun 394:947–954

Matsuda K, Ohga N, Hida Y, Muraki C, Tsuchiya K, Kurosu T, Akino T, Shih SC, Totsuka Y, Klagsbrun M, Shindoh M, Hida K (2011) HuR keeps an angiogenic switch on by stabilising mRNA of VEGF and COX-2 in tumour endothelium. Br J Cancer 104:819–829

Merrick WC (2004) Cap-dependent and cap-independent translation in eukaryotic systems. Gene 332:1–11

Mpekris F, Baish JW, Stylianopoulos T, Jain RK (2017) Role of vascular normalization in benefit from metronomic chemotherapy. Proc Natl Acad Sci USA 114:1994–1999

Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A (2001) Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50–56

Murakami J, Li TS, Ueda K, Tanaka T, Hamano K (2009) Inhibition of accelerated tumor growth by blocking the recruitment of mobilized endothelial progenitor cells after chemotherapy. Int J Cancer 12:1685–1692

Murakami H, Ogata Y, Akagi Y, Ishibashi N, Shirouzu K (2011) Circulating endothelial progenitor cells in metronomic chemotherapy using irinotecan and/or bevacizumab for colon carcinoma: study of their clinical significance. Exp Ther Med 2:595–600

Natale G, Bocci G (2018) Does metronomic chemotherapy induce tumor angiogenic dormancy? A review of available preclinical and clinical data. Cancer Lett 432:28–37

Nissinen TA, Degerman J, Räsänen M, Poikonen AR, Koskinen S, Mervaala E, Pasternack A, Ritvos O, Kivelä R, Hulmi JJ (2016) Systemic blockade of ACVR2B ligands prevents chemotherapy-induced muscle wasting by restoring muscle protein synthesis without affecting oxidative capacity or atrogenes. Sci Rep 6:32695

Panigrahy D, Kaipainen A, Butterfield CE, Chaponis DM, Laforme AM, Folkman J, Kieran MW (2010) Inhibition of tumor angiogenesis by oral etoposide. Exp Ther Med 1:739–746

Pasquier E, Kavallaris M, Andre N (2010) Metronomic chemotherapy: new rationale for new directions. Nat Rev Clin Oncol 7:455–465

Rao N, Lee YF, Ge R (2015) Novel endogenous angiogenesis inhibitors and their therapeutic potential. Acta Pharmacol Sin 36:1177–1190

Räsänen M, Degerman J, Nissinen TA, Miinalainen I, Kerkelä R, Siltanen A, Backman JT, Mervaala E, Hulmi JJ, Kivelä R, Alitalo K (2016) VEGF-B gene therapy inhibits doxorubicin-induced cardiotoxicity by endothelial protection. Proc Natl Acad Sci USA 113:13144–13149

Rocha GZ, Dias MM, Ropelle ER, Osório-Costa F, Rossato FA, Vercesi AE, Saad MJ, Carvalheira JB (2011) Metformin amplifies chemotherapy-induced AMPK activation and antitumoral growth. Clin Cancer Res 17:3993–4005

Roviello G, Bachelot T, Hudis CA, Curigliano G, Reynolds AR, Petrioli R, Generali D (2017) The role of bevacizumab in solid tumours: a literature based meta-analysis of randomised trials. Eur J Cancer 75:245–258

Russell JS, Brown JM (2014) Circulating mouse Flk1 +/c-Kit +/CD45- cells function as endothelial progenitors cells (EPCs) and stimulate the growth of human tumor xenografts. Mol Cancer 13:177

Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH (2006) Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 355:2542–2550

Sarkar A, Hochedlinger K (2013) The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. Cell Stem Cell 12:15–39

Schmidt KM, Hellerbrand C, Ruemmele P, Michalski CW, Kong B, Kroemer A, Hackl C, Schlitt HJ, Geissler EK, Lang SA (2017) Inhibition of mTORC2 component RICTOR impairs tumor growth in pancreatic cancer models. Oncotarget 8:24491–24505

Shi H, Jiang J, Ji J, Shi M, Cai Q, Chen X, Yu Y, Liu B, Zhu Z, Zhang J (2014) Anti-angiogenesis participates in antitumor effects of metronomic capecitabine on colon cancer. Canc Lett 349:128–135

Simkens LH, van Tinteren H, May A, ten Tije AJ, Creemers GJ, Loosveld OJ, de Jongh FE, Erdkamp FL, Erjavec Z, van der Torren AM, Tol J, Braun HJ, Nieboer P, van der Hoeven JJ, Haasjes JG, Jansen RL, Wals J, Cats A, Derleyn VA, Honkoop AH, Mol L, Punt CJ, Koopman M (2015) Maintenance treatment with capecitabine and bevacizumab in metastatic colorectal cancer (CAIRO3): a phase 3 randomised controlled trial of the Dutch Colorectal Cancer Group. Lancet 385:1843–1852

St Croix B, Rago C, Velculescu V, Traverso G, Romans KE, Montgomery E, Lal A, Riggins GJ, Lengauer C, Vogelstein B, Kinzler KW (2000) Genes expressed in human tumor endothelium. Science 289:1197–1202

Stoelting S, Trefzer T, Kisro J, Steinke A, Wagner T, Peters SO (2008) Low-dose oral metronomic chemotherapy prevents mobilization of endothelial progenitor cells into the blood of cancer patients. Vivo 22:831–836

Trevisani G, Bagnardi V, Sangalli C, Montagna E, Dellapasqua S, Sporchia A, Iorfida M, Viale G, Barberis M, Veronesi P, Luini A, Intra M, Goldhirsch A, Colleoni M (2015) Phase II study with epirubicin, cisplatin, and infusional fluorouracil followed by weekly paclitaxel with metronomic cyclophosphamide as a preoperative treatment of triple-negative breast cancer. Clin Breast Cancer 15:259–265

Trevisani F, Brandi G, Garuti F, Barbera MA, Tortora R, Casadei Gardini A, Granito A, Tovoli F, De Lorenzo S, Inghilesi AL, Foschi FG, Bernardi M, Marra F, Sacco R, Di Costanzo GG (2018) Metronomic capecitabine as second-line treatment for hepatocellular carcinoma after sorafenib discontinuation. J Cancer Res Clin Oncol 144:403–414

Upreti M, Jyoti A, Sethi P (2013) Tumor microenvironment and nanotherapeutics. Transl Cancer Res 2:309–319

Vacca A, Iurlaro M, Ribatti D, Minischetti M, Nico B, Ria R, Pellegrino A, Dammacco F (1999) Antiangiogenesis is produced by nontoxic doses of vinblastine. Blood 94:4143–4155

Vadysirisack DD, Baenke F, Ory B, Lei K, Ellisen LW (2011) Feedback control of p53 translation by REDD1 and mTORC1 limits the p53-dependent DNA damage response. Mol Cell Biol 31:4356–4365

Visvader JE, Lindeman GJ (2008) Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 8:755–768

Wang S, Lu J, You Q, Huang H, Chen Y, Liu K (2016) The mTOR/AP-1/VEGF signaling pathway regulates vascular endothelial cell growth. Oncotarget 7:53269–53276

Wei J, Jiang H, Gao H, Wang G (2016) Blocking mammalian target of rapamycin (mTOR) attenuates HIF-1α pathways engaged-

vascular endothelial growth factor (VEGF) in diabetic retinopathy. Cell Physiol Biochem 40:1570–1577

Woo IS, Jung YH (2017) Metronomic chemotherapy in metastatic colorectal cancer. Cancer Lett 400:319–324

Yu JX, Huang XF, Lv WM, Ye CS, Peng XZ, Zhang H, Xiao LB, Wang SM (2009) Combination of stromal-derived factor-1α and vascular endothelial growth factor gene-modified endothelial progenitor cells is more effective for ischemic neovascularization. J Vasc Surg 50:608–616

Yuan F, Chen Y, Dellian M, Safabakhsh N, Ferrara N, Jain RK (1996) Time-dependent vascular regression and permeability changes in established human tumor xenografts induced by an anti-vascular endothelial growth factor/vascular permeability factor antibody. Proc Natl Acad Sci USA 93:14765–14770

Yuan F, Shi H, Ji J, Cai Q, Chen X, Yu Y, Liu B, Zhu Z, Zhang J (2015) Capecitabine metronomic chemotherapy inhibits the proliferation of gastric cancer cells through anti-angiogenesis. Oncol Rep 33:1753–1762

Zhang J, Yang PL, Gray NS (2009) Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer 9:28–39