

Review

Antiangiogenic and radiotherapy for cancer treatment

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Summary. Tumor growth and progression depends on tumor angiogenesis, the growth of tumor blood vessels, therefore, targeting tumor angiogenesis is a very promising approach for controlling tumor growth and/or causing regression. Tumor blood vessels have been recognized as a critical component of radiation response to the point of being independent of tumor oxygenation during radiation. An anti-angiogenic approach has been considered less likely to develop drug resistance. But recent findings suggest that anti-angiogenesis causes hypoxia that selects tumor cells (due to genetic instability) that are less dependent on blood supply and leads to drug resistance. The approach of combination of anti-angiogenesis with ionizing radiation by targeting both endothelial and tumor cells should minimize this possibility. The combination may produce a synergistic anti-tumor effect.

Key words: Angiogenesis, ionizing radiation, endothelial cell, VEGF and angiogenic growth factor

Introduction

The formation of new blood vessels in tumors from the existing vessels is essential for the growth of the tumor and metastasis; thus, controlling tumor growth and/or causing regression by focusing on the tumor endothelium remain the subjects of intense investigation. The deprivation of blood supply has shown promising results in preclinical mouse studies; however an overall survival benefit of antiangiogenic therapy alone has not been clinically demonstrated (Miller et al., 2005; Jain et al., 2006). In spite of negative clinical outcomes, a combination of antiangiogenic therapy and cytotoxic therapy has shown promising results over the past few years (Hurwitz et al., 2004; Jain et al., 2006). In 2004, a significant clinical outcome (Hurwitz et al., 2004) led to

the approval of the first antiangiogenic agent, bevacizumab (a humanized anti-VEGF-A antibody), for metastatic colorectal cancer treatment in combination with chemotherapy. Chemotherapeutic drugs target not only the tumor cells but also vascular forming cells. This further validates the importance of antiangiogenic therapy in oncology. Besides antiangiogenic therapy, radiotherapy has been proven to be effective in various tumors; yet, in many cases radiation cannot control local tumor. Despite many improvements in treatment planning, the total radiation dose is often too low for cure due to the risk of normal tissue damage. A combination of traditional radiotherapy and newly validated antiangiogenesis therapy may lead to a better clinical efficacy in various cancers.

Vascular endothelium has been recognized as a critical component of radiation response to the point of being independent of tumor oxygenation during radiation (Folkman and Camphausen, 2001; Paris et al., 2001; Garcia-Barros et al., 2003). An anti-angiogenic approach has been considered less likely to develop drug resistance. But recent findings suggest that anti-angiogenesis therapy causes hypoxia that selects tumor cells (due to genetic instability) that are less dependent on blood supply and leads to drug resistance (Rak et al., 2002; Yu et al., 2002). Therefore, a combination of anti-angiogenesis with irradiation (IR), which targets both endothelial and tumor cells should minimize this possibility. In addition, targeting angiogenic active tumor vessels has very limited cytotoxicity compared to conventional approaches. Finally, antiangiogenic treatment could enhance endothelial and potentially tumor cell responses to radiotherapy, thus enabling to lower radiation doses and minimize tissue damage.

Indeed, in preclinical animal models, a combination of angiogenesis inhibitors with radiation therapy (Mauceri et al., 1998b; Li et al., 2005), or chemotherapy (Teicher et al., 1992; Herbst et al., 1998) shows synergetic therapeutic benefits over antiangiogenic therapy or radiotherapy alone. In clinical studies, a combination of antiangiogenic agents and chemotherapy shows encouraging results as well (Herbst et al., 2002,

2003). In this review, studies of the combination therapy of antiangiogenic agents with radiation in the past decade are discussed. Specifically, key angiogenic promoters, such as vascular endothelial growth factor receptor (VEGFR), epidermal growth factor receptor (EGFR) and other tyrosine kinase receptors are discussed. Moreover, other biological alterations due to irradiation such as microenvironment changes and apoptosis are discussed as well.

Mechanisms of ionizing radiation-induced EC death

IR induces cell death mainly through two mechanisms: clonogenic death and apoptosis. Clonogenic death is also called reproductive death. Cells lose reproductive ability and can't sustain indefinite proliferation to form a large number of progeny. This is true even though the cell may still be physically present and apparent intact, may be able to make proteins and DNA, and even struggle through a few mitoses. This lethal action involves the cell nucleus as the target. IR induces DNA double strand breaks and genetic instability. The cells are unable to repair or incorrectly repair the DNA damage, and lead to cell death. Lethal mutations or dysfunctional chromosomal aberrations are also believed to cause cell death. Combination of IR with angiostatin induces endothelial cell (EC) death mainly through clonogenic cell death (Mauceri et al., 1998a).

Recent studies also demonstrate that IR induces EC death through apoptosis (Geng et al., 2001a; Paris et al., 2001; Garcia-Barros et al., 2003). Although some cell types may be inherently resistant to radiation-induced apoptosis, in other cells, it has been suggested that sensitivity to radiation-induced apoptosis may vary according to the microenvironment. Constant exposure to radiation changes the sensitivity of cells to radiation (Meyn et al., 1994). Basic fibroblast growth factor (bFGF) inhibits radiation-induced apoptosis in ECs (Fuks et al., 1994). VEGF has also been reported to have the ability to block IR induced EC apoptosis, and blocking VEGF signaling sensitized ECs to IR (Geng et al., 2001a). Activation of anti apoptotic signal transduction pathways can prevent radiation-induced cell death. These inherent or altered radioresistance can impair the effectiveness of radiotherapy (Moeller et al., 2005; Truman et al., 2005); in contrary, activation of apoptotic gene and pathway may enhance tumor vascular radiosensitivity.

Activation of some endogenous molecules can also enhance apoptosis and inhibit angiogenesis. Caspase-9 is a member of caspase family of cysteine proteases that initiates a pro-apoptotic cascade by activating downstream caspases such as caspase-3, -6, and -7 (Kuida, 2000). Overexpression of caspase-9 coupled with radiation has a synergistic effect on the inhibition of glioma invasion (Yanamandra et al., 2004). Another endogenous agent, Sphingosine-1-phosphate (S1P), has gained attention as a therapeutic target as well (Toman et

al., 2001). S1P is one of the metabolites of a lipid second messenger, ceramide, and has been shown to regulate various biological functions, such as apoptosis, proliferation, chemotaxis, and angiogenesis (Pyne and Pyne, 2000). The level of ceramide is increased following IR (Vit and Rosselli, 2003), and S1P has been shown to play a role in radiation-induced apoptosis (Morita et al., 2000). Nitric oxide (NO) has many characteristics including cytotoxicity, radiosensitization and anti-angiogenesis. Tumors treated with iNOS gene transfer show large areas of necrosis and abundant apoptosis; furthermore, combination of inducible NO synthases (iNOS) gene transfer with radiation results in a dramatic growth delay (Worthington et al., 2002). Taken together, activation of apoptotic pathways have enhanced the effect of IR. More studies are needed to further explore the therapeutic potential in the near future.

Receptor tyrosine kinase (RTK) in vascular therapy

Of the molecular mechanisms identified to date, activation of endothelial receptor tyrosine kinases by polypeptide growth factors, appears to play a pivotal role in blood vessel growth and function (Conway et al., 2001; Yancopoulos et al., 2000b; Carmeliet, 2003). Indeed, growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and platelet derived growth factor (PDGF) and the ephrins have shown to modulate angiogenesis suggesting that signaling by multiple RTKs is required for the proper assembly of blood vessels.

EGF signaling is a critical mediator of cell division, migration, and survival. The importance of EGF in the development and progression of many solid tumors is well understood. Elevated expression of EGF receptor (EGFR) is associated with enhanced tumor invasion, resistance to chemotherapy and decreased patient survival. Expression of EGF/EGFR is negatively correlated with survival after IR treatment (Barker et al., 2001; Magne et al., 2001). Furthermore, EGF stimulates VEGF production (Goldman et al., 1993), which could modulate angiogenesis.

EGF/EGFR has been shown to have important roles in the response of tumors to IR. Studies have shown that IR activates EGFR signaling and causes radioresistance in tumors (Dent et al., 1999; Sturla et al., 2005); thus, inhibition of EGFR activation has been investigated. Treatment of A431 squamous cell carcinoma with ZD1839 (Iressa), an anti EGFR inhibitor, in vitro reduces proliferation, increases apoptosis, and reduces clonogenic survival after radiation. A strikingly greater than additive effect of ZD1839 in combination with IR on tumor growth delay is observed in vivo. ZD1839 reduces tumor vascularity, as well as levels of VEGF protein and mRNA induced by stimulation with EGF (Solomon et al., 2003). Tumor xenograft studies demonstrate complete regression of both newly

established (20 mm³) and well-established (100 mm³) squamous cell carcinoma when they are treated with the combination of EGFR neutralizing antibody, IMC-C225, and radiation (Huang and Harari, 2000). Clinical efficacy of IMC-C225 with radiation appears to involve multiple anticancer mechanisms, including inhibition of cell cycle progression, accumulation of cells in G1, G2-M radiosensitive phase, induction of apoptosis, reduction in tumor angiogenesis markers such as VEGF and factor VIII, inhibition of metastasis and its ability to enhance the response to chemotherapy and radiation therapy (Huang and Harari, 2000; Herbst et al., 2001). The safety of combination therapy of EGFR antibody, cetuximab, and IR was shown in phase I clinical trial (Robert et al., 2001).

PDGF/PDGFR signaling plays a critical role in tumor development. PDGF stimulates growth of cancer cells in an autocrine fashion, and stromal cells and angiogenesis in a paracrine fashion. PDGF has been shown to regulate EC-induced recruitment of vascular smooth muscle cells (SMCs) and vascular maturation (Bergers et al., 2003). Inactivation of PDGF-B in transgenic mice displays vascular defects with loss of pericytes/SMCs (Lindahl et al., 1997). PDGF/PDGFR expression has been noted in various neoplasms, and many drugs to inhibit PDGFR are in various stages of clinical studies (Joyce et al., 2003; Board and Jayson, 2005). In a preclinical animal tumor study, a combination of VEGFR inhibitor and PDGFR inhibitor showed stronger efficacy than either single agent (Bergers et al., 2003). Currently, studies of the combination of PDGFR inhibitor and radiation are limited. One study using Imatinib mesylate (Gleevec[®]), an inhibitor of catalytic activity of the Abl, PDGFR and c-kit tyrosine kinases, showed that it radio-sensitized glioblastoma (RuSi RS1) by specifically inhibiting the phosphorylation of PDGFR- β (Holdhoff et al., 2005). More studies need to be conducted to carefully examine the effects of this combination in cancer therapy.

Endothelial specific RTKs in vascular therapy

Despite the role of a variety of growth factors in angiogenesis, the expression of their cognate RTKs outside of the vasculature has dampened enthusiasm for these pathways as therapeutic targets. Importantly, RTKs for two families of angiogenic growth factors, the vascular endothelial growth factor family (VEGF) and the angiopoietin (Ang) family, are expressed predominantly on vascular endothelial cells, making them attractive targets for both pro- and anti-angiogenic therapy (Yancopoulos et al., 2000a; Ferrara et al., 2003).

The importance of VEGF in angiogenesis has been studied extensively (Ferrara et al., 2003). The VEGF pathway is required for embryonic vascular development as well as tumor angiogenesis (Kim et al., 1993; Millauer et al., 1996; Lin et al., 1998b). VEGF mediates the survival of vascular endothelium (Ferrara, 2000; Matsumoto and Claesson-Welsh, 2001). Blocking the

VEGF signaling enhances EC death and tumor growth inhibition when combined with radiation (Gorski et al., 1999a; Geng et al., 2001a; Kozin et al., 2001a; Ning et al., 2002). Furthermore, high level of VEGF is associated with poor prognosis and poor therapeutic outcomes (Valter et al., 1999). Thus, a lot of attention has been gained to inhibit the signaling pathway downstream of VEGF/VEGFR. High dose of IR inhibits angiogenesis by inhibiting EC survival, proliferation, migration and tube formation. However, low dose of IR can also induce VEGF production and protect tumor blood vessels, resulting in tumor radioresistance (Gorski et al., 1999b). Due to the importance of VEGF in tumor development and radiotherapy, many therapeutic agents against VEGF/VEGFR signaling have been developed: neutralizing antibodies against VEGF and VEGFR, as well as various VEGFR tyrosine kinase chemical inhibitors. A major clinical breakthrough using a VEGF inhibitor, bevacizumab, came in 2003 in metastatic renal-cell cancer. Bevacizumab significantly prolongs the time of free of tumor progression, yet there is no difference in survival in the end. Combination of VEGF inhibitor with other treatment was suggested (Yang et al., 2003).

Inhibition of VEGF/VEGFR signaling was examined using various forms of inhibitors: antibody, adenovirus, and small molecule inhibitors. VEGF signaling is mainly transduced through two receptors (VEGFR1 and VEGFR2) in angiogenesis (Ferrara et al., 2003). Administration of VEGFR2-blocking antibodies increases the response to IR both in EC culture and in vivo animal studies, suggesting that molecular inhibition of VEGFR2 alone, and in combination with radiation, can enhance tumor response through molecular targeting of tumor vasculature (Li et al., 2005). Another study using VEGF antibody shows that inhibition of VEGF improved radiation response (Lee et al., 2000; Kozin et al., 2001b). We observed an increase of radio response in radioresistant glioma using viral vector expressing soluble VEGFR2 in vivo (Geng et al., 2001b). Furthermore, small molecule inhibitors have been developed and tested. SU5416 and SU6668, VEGFR inhibitors, enhance the antiangiogenic effects of IR on cultured ECs. Another VEGF inhibitor, PTK787/ZK222584, with IR was shown to abrogate VEGF dependent proliferation of HUVEC in a dose dependent manner (Hess et al., 2001). Using the same inhibitor, SW480 colorectal tumors from animals that received a combined treatment regimen, display not only a significant decrease in the number of microvessels in the tumor xenograft but also an extended tumor growth delay. Other RTK inhibitors such as SU11248 and anginex, also show promising vasculature destruction and tumor control when combined with radiation in animal tumor models (Schueneman et al., 2003; Dings et al., 2005). Besides the combination of VEGF or broad RTK inhibitor with IR, triple combination of IR, chemotherapy (pemetrexed), and VEGFR inhibitor (e.g. SU5416, SU11657) has shown promising results as well

(Bischof et al., 2004; Huber et al., 2005).

The second endothelial specific RTK is the Tie family (Tie1 and Tie2). Both Tie1 and Tie2 signaling is required for embryonic vascular development (Dumont et al., 1994; Sato et al., 1995). Currently Tie1 remains an orphan receptor although some studies indicate angiopoietins, the ligands for Tie2 receptor, also bind Tie1 (Saharinen et al., 2005). Multiple angiopoietins (Ang) have been identified (Davis et al., 1996; Suri et al., 1996; Maisonpierre et al., 1997; Valenzuela et al., 1999). Ang1, an agonist, stimulates Tie2 phosphorylation in ECs. Ang2 has been considered to be an antagonist of Tie2 that blocks Tie2 activation induced by Ang1 in ECs. Disrupting the function of Ang1 or overproduction of Ang2 yielded a phenotype similar to the Tie2 knockout confirming the importance of the Ang/Tie2 pathway during embryonic vascular development (Suri et al., 1996; Maisonpierre et al., 1997). The genetic evidence suggests that the VEGF pathway and the Tie2 pathway seem to work in a complementary and coordinated fashion during vascular development, with VEGF and Ang2 acting during the early stages of vessel development (Shalaby et al., 1995; Carmeliet et al., 1996; Ferrara et al., 1996), and Ang1 acting later to promote angiogenic remodeling as well as vessel maturation and maintenance (Sato et al., 1995; Suri et al., 1996).

Tie2 has been shown to play a critical role in tumor angiogenesis. Tie2 and its ligands (Ang1, 2) are upregulated in tumors compared to normal tissue (Peters et al., 1998; Stratmann et al., 1998; Takahama et al., 1999; Zagzag et al., 1999; Caine et al., 2003). Blocking Tie2 activation inhibited tumor angiogenesis and tumor growth in vivo (Lin et al., 1997; Zadeh et al., 2004). Systemic delivery of a soluble Tie2 receptor (ExTek) using an adenoviral vector inhibited the growth of both well-established primary tumors and tumor metastases (Lin et al., 1998a). Blocking either the Tie2 or VEGF pathway for the mammary tumor significantly inhibited tumor angiogenesis and tumor growth (Lin et al., 1997, 1998b). These results suggest that Tie2 and VEGF are two independent mechanisms essential for mammary tumor angiogenesis.

The role of Ang1 in tumor development remains controversial. Ang1 was shown to either inhibit or promote tumor growth (Hayes et al., 2000; Hawighorst et al., 2002; Stoeltzing et al., 2002, 2003), revealing the complexity of Ang1 in angiogenesis and suggesting that the role of Ang1 in tumor progression is context dependent. Elevated expression of Ang1 and Ang2 was observed in various cancers (Mitsuhashi et al., 2003; Moon et al., 2003; Zhao et al., 2003; Caine et al., 2004; Ogawa et al., 2004; Sun et al., 2004). The limited number of studies regarding Tie2 and angiopoietins with IR showed that Ang-1 protects ECs from IR-induced apoptosis (Kwak et al., 2000; Cho et al., 2004). Collectively, these data support a critical role of Ang/Tie2 signaling in tumor angiogenesis and potential vascular survival and radiation resistance.

Impacts of the tumor microenvironment on tumor vascular response to IR

Tumor blood vessels are structurally and functionally abnormal (Carmeliet and Jain, 2000). Most of them are dilated, tortuous with extensive fenestrations and branching. This abnormal organization causes variable blood flow and vascular leakiness, and prevents the uniform delivery of circulating chemotherapeutic agents and oxygen (a radiotherapy enhancer). One of the recent fascinating findings demonstrates that antiangiogenic treatment "normalizes" tumor blood vessels by reducing tumor vascular tortuosity and vascular permeability, and improving blood flow. This normalization may be occurring by pruning the immature and inefficient blood vessels (Jain, 2001), as a result it will improve blood flow and oxygenation in tumors. This could explain the enhanced tumor response to radiotherapy when combined with antiangiogenic therapy. As well known, hypoxia induces angiogenic factor expression and angiogenesis. As one would expect, a potent hypoxia selective cytotoxin, Quinoxaline 1,4-dioxide (DCQ), inhibits the formation of tube-like structures of ECs and significantly reduces neoangiogenesis in vivo. When combined with radiation, DCQ delays tumor growth and reduces mean tumor volume (Gali-Muhtasib et al., 2004). In another study, an inhibition of the biological pathways of small GTPase Rho improved U87 glioblastoma oxygenation by inducing more homogeneous distribution of vessels. Improvement of the tumor oxygenation with a significant decrease of the vessel density and of the matrix metalloproteinase-2 (MMP-2) expression has led to the radiosensitization of tumors (Ader et al., 2003).

Studies also found that tumor microvasculature response to radiotherapy is heterogeneous (Dewhirst et al., 1990; Lin, 2003). Extracellular matrix (ECM) is known to regulate nearly all cellular processes during vessel formation. Type IV collagen is thought to be remodeled by proteolytic enzymes during the initiation of angiogenesis. Cryptic epitopes of collagen Type IV are differentially exposed during matrix remodeling and are key mediators of angiogenesis. MMPs play a major role in degradation of ECM including collagen IV. Among various MMPs, MMP-2 and -9 seem to play a key role in tumor cell invasion, metastasis, and angiogenesis. Microenvironment changes induced by irradiation are noted in malignant glioma that are highly vascularized, invasive, and resistant to radiotherapy. Irradiation of C6 glioma cells decreases cell proliferation in a dose-dependent manner in vitro; however, it enhances angiogenic activity in vivo, possibly by the induction of expression and secretion of MMP-2 and MMP-9 (Parthymou et al., 2004). In spite of the promising results of MMP inhibitor (MPI) as a therapeutic agent, all the clinical trials using MPI have failed to exert any significant effects (Coussens et al., 2002). This implies a very complex role of MMPs in vascular development and tumor progression.

In the past decade, the importance of the tumor microenvironment was revealed greatly, and our knowledge of tumors has expanded from a mass of neoplastic cells to a complex tissue. Further knowledge of the neoplastic environment should lead to more success in clinical trials.

Other common agents that possess antiangiogenic function

Combination of paclitaxel, antimicrotubule agent, and IR is commonly used in clinic. Paclitaxel amplifies the cytotoxic effects of IR *in vitro*, presumably by inducing cell cycle arrest at metaphase, which is known to be a very radiosensitive phase of the cell cycle. Paclitaxel inhibits EC proliferation, migration, and tube formation (differentiation) at one-tenth the concentration needed to achieve a similar effect on tumor cell lines. In combination with radiation, inhibition of EC function is shown to be additive (Dicker et al., 2003). Heterogeneous responses were also observed in different types of tumors. In colorectal cancer cells, paclitaxel functions as a radiosensitizer (Chendil et al., 2000); yet, in breast cancer cells, IR is shown to antagonize the antitumor activity of paclitaxel (Sui et al., 2004).

ZD6126, an anti tubulin agent, selectively affects the morphology of proliferating and immature endothelial cells by disrupting the tubulin cytoskeleton. ZD6126 reduces tumor blood vessels, and increases tumor necrosis. Those effects are more extensive with increasing tumor size. When combined with radiation, ZD6126 treatment results in little enhancement of the antitumor effect of radiation in small tumors but markedly increases cell death in tumors larger than 1.0 g (Siemann and Rojani, 2005). This finding is interesting and different from VEGF/VEGFR inhibitor, which works only when the tumor is small (Zips et al., 2005).

One of the most studied antiangiogenic agent, thalidomide, is shown to neutralize two potent angiogenic factors, basic fibroblast growth factor (bFGF) (D'Amato et al., 1994) and VEGF (Kruse et al., 1998). It also induces microenvironmental changes; the vessels are more evenly distributed throughout the tumor in controls than the treated group. These observed microenvironmental changes induced by thalidomide are sufficient to radiosensitize tumors (Ansiaux et al., 2005). It is also suggested that thalidomide exerts antiangiogenic effect by producing reactive oxygen species (ROS).

Timing of angiogenic therapy

The degree of sensitivity of ECs to radiation-induced damage may depend on the growth/survival factors in the tumor microenvironment. It also depends on the dose and time interval of the treatment. In the combination therapy regimens, timing of the drug administration has been explored to determine the most effective regimen. Many studies show that IR following antiangiogenic treatment sensitizes ECs and enhances the effect of

radiotherapy. Constant administration of SU11248 (a general RTK inhibitor) beyond the completion of radiotherapy results in better tumor control (Schueneman et al., 2003). Regimen with radiotherapy given after the institution of SU11657 (VEGFR1), a multi targeted small molecule inhibitor of VEGFR and PDGFR, is more effective than radiotherapy preceding SU11657 treatment. Inhibition of Akt signaling by SU11657 may result in normalization of tumor blood vessels that causes prerequisite physiologic conditions for subsequent radio/chemotherapy and direct sensitization of EC to radio/chemotherapy (Huber et al., 2005). Injection of antiangiogenic agent, anginex, at a specific time point prior to radiation treatment sensitizes ECs to radiation and significantly prolongs radiation-induced tumor growth delay (Dings et al., 2005). SU5416 (VEGFR-2 inhibitor) given before irradiation shows a pronounced increase in radiosensitivity (Geng et al., 2001b). Thalidomide treatment significantly increases the tumor regrowth delay when applied during 2 days before the irradiation (Ansiaux et al., 2005). On the other hand, irradiation of the host before tumor transplantation has enhanced susceptibility of irradiated vessels to PTK787/ZK22258 (Zips et al., 2005).

Metronomic chemotherapy which gives treatment in small doses on frequent schedule for prolonged periods without interruption seemed to work better than conventional chemotherapy which gives maximum tolerated doses with 2-3 weeks of breaks between therapies. The optimum biological dose for the metronomic chemotherapy was correlated with the reduction of VEGFR2 positive circulating endothelial precursors (Shaked et al., 2005). Combination of IR and metronomic antiangiogenic treatment may enhance the efficacy further. Clinical trials for better therapeutic regimens to enhance efficacy and lessen side effects are under way (Kerbel and Kamen, 2004).

Endothelial progenitors and vasculogenesis

Vascular growth is a physiologic process as well as a pathologic process after injury. A high dose of radiation treatment damages vascular networks and reduces blood flow. However, the vascular endothelium may be repaired and blood flow may be restored after the treatment. Understanding the mechanism of vascular regrowth is important for cancer therapy since tumor recurrence depends on tumor vascular regrowth. Vascular formation can be accomplished in two ways, angiogenesis and vasculogenesis. Angiogenesis is defined as capillary sprouting from surrounding preexisting vascular network (Risau, 1997). Vasculogenesis is a *de novo* process, in which the vascular network arises from the *in situ* differentiation and proliferation of angioblasts to form the primary capillary networks (Risau and Flamme, 1995). Recent findings demonstrate the existence of endothelial progenitor cells (EPCs) derived from bone marrow and these EPCs contribute to adult vascular formation

(Asahara et al., 1997, 1999; Rafii, 2000; Rafii and Lyden, 2003). VEGF induces an acute mobilization of EPCs in vivo (Hattori et al., 2001; Moore et al., 2001). In contrast, over-expression of Ang1 induces a delayed mobilization of bone marrow derived-EPCs (BM-EPCs). As expected, angiogenic inhibitors such as endostatin (Capillo et al., 2003; Schuch et al., 2003), angiostatin (Ito et al., 1999), and VEGFR2 inhibitor (ZD6474) (Beaudry et al., 2005) hinder EPC mobilization.

Vasculogenesis also plays a role in tumor neovascularization. A study shows that transplantation of a normal bone marrow to Id1/Id3 knockout mice, which lack vascular formation ability, restores their ability to form tumor blood vessels (Lyden et al., 2001). The data suggest vasculogenesis or BM-EPCs are sufficient to form tumor blood vessels in adult transgenic mice. Vasculogenesis contributes to tumor blood vessel formation in a variety of tumors (de Bont et al., 2001; Dwenger et al., 2004; Shirakawa et al., 2002). Based on this thinking, treatment schemes to eliminate EPCs in tumor vascular repair should prevent or reduce tumor regrowth and recurrence. Indeed, a study shows a prolonged tumor control by SU11248 treatment beyond the completion of radiotherapy (Schueneman et al., 2003). There is a need to carefully observe the fate and the importance of EPC following IR. Furthermore, whether EPC is recruited into tumor vasculature is still controversial (De Palma et al., 2003; Peters et al., 2005). A difference in the extent of the recruitment of EPC between mice and humans is also noted (Peters et al., 2005). Further studies need to be done to clarify the importance of EPCs in tumor vascular formation in human. Nevertheless, control of EPC mobilization can be used as an adjuvant therapy following the combination of antiangiogenic therapy and IR.

Conclusions

Taken together, antiangiogenic agents are more effective when combined with IR often by enhancing the radiosensitivity. Some of the widely used drugs such as paclitaxel (Dicker et al., 2003), ZD6126 (Siemann and Rojiani, 2005), and thalidomide (Ansiaux et al., 2005) have shown to have antiangiogenic activities and radiosensitize tumors. Polyunsaturated fatty acids and 2-methoxyoestradiol (estrogen metabolite) are speculated to exert antiangiogenic effects and enhance radiosensitivity as well (Amorino et al., 2000; Das, 2002). Many of the antiangiogenic agents work in different aspects of tumor biology. Some antiangiogenic agents such as VEGFR inhibitors work better when the tumor is smaller and others such as ZD6126 work better against large tumors. Some agents such as C225 work regardless of the size of the tumor. Besides the size of tumors, unfavorable microenvironment factors such as resistance to apoptosis can be removed by certain antiangiogenic agents. Further studies of EPC may provide a new therapeutic approach. The development of novel antiangiogenic agents would advance in the cancer

treatment. More importantly, finding better combinations of treatment depending on the needs of each tumor and also best planning hold great promise for cancer treatment.

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Antiangiogenic and radiation cancer therapy

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Antiangiogenic and radiation cancer therapy

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