Antiangiogenic therapy: a novel approach to overcome tumor hypoxia

Fang Peng$^{1,2}$, Ming Chen$^{1,2}$

$^{1}$ State Key Laboratory of Oncology in South China, Guangzhou, Guangdong 510060, P. R. China; $^{2}$ Department of Radiation Oncology, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong 510060, P. R. China

Abstract Hypoxia is a common phenomenon in solid tumors. Resistance of hypoxic tumor cells to radiation is a significant reason of failure in the local control of tumors. The growth and metastasis of solid tumors rely on blood vessels. Antiangiogenic agents mainly target tumor blood vessels, and radiation therapy mainly targets tumor cells. Combination of antiangiogenic treatment and radiation exhibits synergistic effect, which improves the response of tumors to radiation therapy. The mechanisms of interaction between antiangiogenic agents and ionizing radiation are complex and involve interactions between tumor cells and tumor microenvironment, including tumor oxygenation, stroma, and vasculature. The original mechanism of antiangiogenesis is to induce ischemia and hypoxia in tumors, thereby, “starve” the tumors. However, recently, emerging data suggest that antiangiogenic agents could reduce the proportion of hypoxic cells through normalizing tumor vasculature, decreasing oxygen consumption, and other mechanisms. The use of antiangiogenic agents provides a new approach to overcome the hypoxia problem, and ultimately improves the efficacy of radiation therapy. In this review, we discuss tumor hypoxia, tumor angiogenesis and its regulation, mechanisms of antiangiogenic therapy combined with radiation therapy, and how antiangiogenic therapy overcomes tumor hypoxia.

Key words: Cell hypoxia, radiotherapy, antiangiogenesis

Introduction

Hypoxia is a common phenomenon in solid tumors, which can reduce the sensitivity of tumor cells to radiotherapy and chemotherapy and, thus, is an important factor affecting cancer therapy. In many tumors, including cervical cancer, head and neck cancer, and soft tissue sarcoma, the more severe hypoxia usually indicates the worse prognosis. Since over 50 years ago when Thomlinson and Gray found the resistance of hypoxic cells to radiation therapy, researchers have been trying to solve the problem of tumor hypoxia. Strategies to overcome tumor hypoxia include: (1) increasing the oxygen level within the tumor (high-pressure oxygen inhalation, increasing hemoglobin level, application of nicotinamide, etc.); (2) elevating blood flow within the tumor; (3) applying hypoxic cell sensitizers; (4) unequal fractionation irradiation therapy; (5) applying biological reducing agents; (6) microwave and thermotherapy; (7) gene therapy; and so on. However, the clinical applications of the above methods to overcome tumor hypoxia are unsatisfied. Tumor hypoxia remains an enormous obstacle in cancer therapy.

Oxygen is supplied via the blood vessels and the distance of oxygen diffusion from the capillary vessels is only 100–200 μm. The abnormality of tumor blood vessels (high disorder, tortuosity, swelling, and excessive branching) leads to chaos of blood flow, hypoxia, and accumulation of acidic materials. Researches on tumor vascular pathology have resulted in antiangiogenic drugs for cancer therapy. The potential advantages of antiangiogenic drugs over conventional chemotherapy include: (1) the targets of antiangiogenic drugs are tumor blood vessels, which makes the drug delivery easy and a higher level of drug accumulation at the target site; (2) antiangiogenic drugs target the newly generated blood vessels, which have...
negligible toxicity on normal tissues; (3) gene expression in vascular endothelial cells is relatively stable, which can rarely induce drug resistance; (4) the angiogenesis-dependent growth is a common characteristic of all tumors, which will make antiangiogenesis a broad-spectrum therapy; (5) antiangiogenesis has specific mechanisms and can combine with other therapies. However, antiangiogenic drugs also have some limitations: (1) because of the slow onset, patients need continuous and long medication; (2) antiangiogenic drugs have no effect on small non-vascular lesions; (3) redundancy of signaling pathways can undermine the efficacy of antiangiogenic drugs; (4) antiangiogenic drugs can not ultimately eliminate cancer stem cells, which stay in a quiescent state and resist cytotoxicity of antiangiogenesis therapy. These characteristics destine antiangiogenesis a low efficiency when used alone. One strategy to address these issues is to combine antiangiogenic therapy with other conventional therapies (radiotherapy and chemotherapy), which can target tumor blood vessels and tumor cells concurrently and ultimately kill tumor tissues. Massive studies have confirmed that single use of antiangiogenic drugs can not bring long-term survival benefit, while combination of antiangiogenesis and radiotherapy can often produce additive or synergistic antitumor effect.

In recent years, many studies indicate that radiation therapy combined with antiangiogenic drugs can improve efficacy[11]. The mechanisms of this synergistic effect are very complex, involving tumor cells themselves and tumor microenvironment (including oxygen supply, matrix, and blood vessels). Clarification of these mechanisms can guide the clinical development of therapeutic protocols combining antiangiogenesis and radiation. This review covers pre-clinical and clinical research findings, and proposes that antiangiogenic therapy is a new strategy to overcome tumor hypoxia and improve the effect of radiation therapy. The combination of angiogenic therapy and radiation therapy may be a new combined therapy for cancer[2].

Tumor hypoxia and radiation therapy

Relevance of oxygen on radiation therapy

Oxygen plays an important role in the generation of free radicals by ionizing radiation and subsequent radiation damage and functions as a key mediator to stabilize radiation injury by ionizing radiation. In the absence of oxygen, more radiation damage is repaired; thereby the effect of radiation is compromised. Radiosensitivity of hypoxic cells is about 1/3 of that of hyperoxic cells. Thus, radiation resistance of hypoxic cells is often the origin of resistance to therapy[2].

Formation of tumor hypoxia

Hyoxia is an important pathophysiologic feature in the microenvironment of solid tumors[2], which is caused by the imbalance between oxygen supply and consumption. The major mechanisms of tumor hypoxia include that (1) disorder and dysfunction of intratumoral microvessels lead to severe restricted oxygen perfusion (i.e. acute hypoxia); (2) relative shortage of blood vessel density in rapidly growing tumor causes restricted oxygen diffusion (i.e. chronic hypoxia); and (3) tumor- or treatment-related anemia reduces the oxygen content in blood (i.e. anemic hypoxia)[2,8].

Consequences of tumor hypoxia

Tumor hypoxia has two-sided functions. On the one hand, hypoxia down-regulates cellular protein synthesis and blocks cell proliferation; most cells stay at G0 phase or even go to apoptosis or necrosis; on the other hand, hypoxia prompts resistance of tumor cells to chemotherapy and radiotherapy, and accelerates tumor progression. Meanwhile, long-term hypoxia leads to the unstable expression of genes regulating survival and apoptosis, increasing mutation probability which can cause tolerance to hypoxia and exacerbate the malignancy. Hypoxia can promote tumor cells to overcome malnutrition and, by proliferation, invasion, pervasion, and metastasis, to evade the harsh microenvironment[11,2].

Tumor angiogenesis and its regulation

The survival, growth, and metastasis of solid tumors are dependent on tumor blood vessels. Tumor will not grow beyond 1–2 mm3 in the absence of blood vessels[11]. Tumor blood vessels have abnormal structure and functions. The structural disorder manifests as increase of microvessel density, tortuous morphology, dilation, multi-branch bifurcation, thin vessel wall with many fractures, abnormal endothelial cells (overlapping growth and breaking into the vessel lumens), abnormal pericytes (loose or missing cellular junction), uneven basement membrane. Such abnormal structure leads to bloodstream disorders, perfusion obstruction, increased leakage and increased interstitial fluid pressure, impeding the delivery of drugs and oxygen[2].
Tumor angiogenesis is the result of imbalance of a variety of pro-angiogenic and antiangiogenic factors\(^{[4]}\). Among these factors, vascular endothelial growth factor (VEGF) is the most important angiogenesis-stimulating factor. VEGF can maintain survival as well as induce proliferation and migration of endothelial cells, recruit bone marrow-derived hematopoietic progenitor or stem cells, and increase vascular permeability\(^{[5]}\). Recent studies found that VEGF is a negative regulator of the function of pericytes and maturation of blood vessels\(^{[6]}\). Hypoxia is the strongest stimulator of VEGF. Under hypoxia, hypoxia-inducible factor 1-alpha (HIF-1\(\alpha\)) transcription is up-regulated and then activates VEGF transcription\(^{[4]}\), promoting the formation of abnormal blood vessels. This vicious cycle further exacerbates tumor hypoxia\(^{[1]}\).

**Antiangiogenic therapy**

In 1971, Folkman first proposed that tumor growth and metastasis were dependent on blood vessels and blocking angiogenesis would be an effective strategy to inhibit tumor growth. Targets of anti-angiogenic therapy include VEGF, vascular endothelial cells, matrix metalloproteinases, integrins, and so on. Antiangiogenic agents can be divided into two categories: (1) antiangiogenic agents (AA) targeting new blood vessels and (2) vascular-damaging agents (VA) targeting existing vessels\(^{[1]}\).

In recent years, more and more antiangiogenic drugs have been developed and some of them have been used in clinic trials. Bevacizumab (Avastin), a recombinant anti-VEGF monoclonal antibody, has been approved by the US FDA for clinical use in 2004. In the phase III clinical trials in patients with colorectal cancer, bevacizumab combined with 5-fluorouracil-based conventional chemotherapy extended the median survival time by 4.7 months\(^{[5]}\). The successful application of bevacizumab confirmed the hypothesis of antiangiogenesis and has tremendous impact on cancer therapy. Now bevacizumab has been approved for treatment of advanced colorectal cancer, non-small cell lung cancer, metastatic renal cell cancer, breast cancer, and glioblastoma. Besides combining with conventional chemotherapy, bevacizumab could combine with radiation therapy safely and effectively\(^{[1]}\). In September 2005, recombinant human endostatin has been approved by China state food and drug administration (SFDA). The phase III clinical trial of endostatin in China also showed that combination of endostatin and NP regimen (vinorelbine and cisplatin) significantly improved the efficacy on advanced non-small cell lung cancer and safely extended the median time of tumor progression\(^{[8,10]}\). It is widely agreed that the effect of antiangiogenic drug alone is very limited\(^{[1]}\). With the success of combination with chemotherapy, antiangiogenic drugs are now combined together with radiation therapy\(^{[1]}\).

**Combination therapy of antiangiogenesis and radiation**

**Possible mechanisms of combination therapy of antiangiogenesis and radiation**

The initial rationale of antiangiogenic therapy is that ischemia and hypoxia cause tumor death by “starvation”. By the same reasoning, antiangiogenesis will cause radiation resistance by reducing the oxygen delivery. However, many experimental studies found that combination of antiangiogenic drugs and radiation can improve cancer treatment. The possible reasons include follows: (1) indirect inhibition of tumor angiogenesis via VEGF and its receptor; (2) direct improvement of sensitivity of endothelial cells to radiotherapy, such as apoptosis of endothelial cells; (3) direct improvement of radiosensitivity of tumor cells, such as apoptosis of tumor cells; (4) decrease the proportion of hypoxic cells and increase oxygen content in tumors.

In summary, the main target of antiangiogenesis is tumor blood vessels and the main target of radiotherapy is tumor cells. There is synergic effect between these two therapies\(^{[1]}\). The following content focused on the fourth possible mechanism.

**Overcoming tumor hypoxia by antiangiogenesis**

Pre-clinical and clinical studies have found that many antiangiogenic drugs could overcome tumor hypoxia and improve tumor response to radiation therapy when administered at early stage\(^{[1]}\). These drugs include: DC101, Bevacizumab, Trastuzumab, Erlotinib, SU5416, Thalidomide, Vandetanib (ZD6474), Anginex, anti-VEGF monoclonal antibody which belong to AA Category; and ZD6126 which belongs to VA Category (Table 1).

DC101 is a specific anti-VEGFR2 monoclonal antibody. Winkler et al.\(^{[12]}\) treated glioma-bearing nude mice with combination therapy of radiation and DC101. Tumor hypoxia began to decrease at the day 1 after DC101 administration, most notably on day 5, and rose on day 8; radiotherapy was applied at day 4-day 6 after DC101 administration, tumor growth was significantly delayed and synergic effect was achieved\(^{[12]}\). Other studies have found that SU5416\(^{[16]}\), Vandetanib\(^{[19]}\), Thalidomide\(^{[20]}\) and Anginex\(^{[15]}\) could increase tumor oxygen to the maximum in 24 hours after treatment.
when structure and function of tumor blood vessels and therapy can produce a specific increasing sensitivity to chemotherapy. Antiangiogenic deliver oxygen and drugs to tumor cells more efficiently, regression, and then paired tumor blood vessels can repair the abnormal vascular system in tumors before regression, and then the repaired tumor blood vessels can deliver oxygen and drugs to tumor cells more efficiently, increasing the sensitivity to chemotherapy. Antiangiogenic therapy can produce a specific “time window”, a break when structure and function of tumor blood vessels and microenvironment temporarily become normalized. The structure of tumor vascular resumes order (uniform distribution and diameter, increased pericyte coverage, and uniform basement membrane). The potential function of the normalization of tumor blood vessels in tumor tissues is to decrease interstitial fluid pressure in tumor tissues and overcome hypoxia temporarily so that antitumor drugs can easily access the inside of tumors, therefore, increase the sensitivity to chemotherapy and improve efficacy.

Besides the antiangiogenic drugs stated in Table 1, many other direct or indirect antiangiogenic drugs, such as TNP-470, Gleevec, Erbitux, Sunitinib, TSU-68, and KRN951 can normalize the tumor vascular system in a variety of animal models. In addition, bevacizumab and cediranib (AZD2171) in human tumors also confirmed the tumor vascular normalization. In the time window of normalization of tumor blood vessels, there are consistence among the improvement of tumor tissue perfusion, overcome hypoxia, improved sensitivity to radiotherapy, and the dynamic changes in tumor morphology. Now studies have identified the genes regulating tumor blood vessels normalization, including regulator of G-protein signalling 5 (RGS5), prolyl hydroxylase domain 2 (PHD2), endothelial nitric oxide synthase (eNOS), angiopoietin-2, and Roundabout 4. RGS5 and PHD2 inhibit the normalization of tumor vessels, whereas eNOS and Robo4 promote this process. A variety of tumor angiogenic factors are closely related to the normalization of tumor vessels, such as VEGF, placenta growth factor (PIGF), epidermal growth factor receptor (EGFR), semaphorin 3A, angiopoetin-1, angiopoetin-2, platelet derived growth factor-C (PDGF-C), and so on.

### Table 1  Antiangiogenic and vascular-targeting agents that improve tumor oxygen tension and radiation response

<table>
<thead>
<tr>
<th>Drug</th>
<th>Increased tumor oxygenation</th>
<th>Enhanced radiation therapy efficacy</th>
<th>Model</th>
<th>Mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>Yes</td>
<td>Yes</td>
<td>U87 human glioma xenografts</td>
<td>Normalized tumor vasculature</td>
<td>[8,13-15]</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Yes</td>
<td>Not studied</td>
<td>Her2+ MCF7 human breast cancer xenografts</td>
<td>Normalized tumor vasculature</td>
<td>[16]</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>Yes</td>
<td>Yes</td>
<td>Mouse tumor xenografts</td>
<td>Normalized tumor vasculature</td>
<td>[17]</td>
</tr>
<tr>
<td>SU5416</td>
<td>Yes</td>
<td>Yes</td>
<td>Mouse liver cancer and FSAll fibrosarcoma xenografts</td>
<td>Inhibited mitochondrial respiration</td>
<td>[18]</td>
</tr>
<tr>
<td>Vandetanib</td>
<td>Yes</td>
<td>Yes</td>
<td>Mouse liver cancer xenografts</td>
<td>Decreased oxygen consumption</td>
<td>[19]</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>Yes</td>
<td>Yes</td>
<td>Mouse FSAll fibrosarcoma xenografts</td>
<td>Normalized tumor vasculature</td>
<td>[20]</td>
</tr>
<tr>
<td>Anginex</td>
<td>Yes</td>
<td>Yes</td>
<td>MA148 human ovarian carcinoma, B16F10 murine melanoma, and SCK murine breast carcinoma xenografts</td>
<td>Normalized tumor vasculature</td>
<td>[15,21]</td>
</tr>
<tr>
<td>Anti-VEGF</td>
<td>Yes</td>
<td>Yes</td>
<td>U87 human glioma xenografts</td>
<td>Not studied</td>
<td>[22]</td>
</tr>
<tr>
<td>ZD6126</td>
<td>No</td>
<td>Yes</td>
<td>Mouse KHT sarcoma xenografts</td>
<td>Not studied</td>
<td>[23,24]</td>
</tr>
</tbody>
</table>

### Mechanisms of overcoming tumor hypoxia by antiangiogenic drugs

The mechanisms by which antiangiogenic drugs overcome tumor hypoxia and the combination therapy of antiangiogenesis and radiation improve efficacy are not fully understood. Jain’s theory of normalization of tumor blood vessels drew the most attention in this field. This theory states that the rational use of antiangiogenic drugs can repair the abnormal vascular system in tumors before regression, and then the repaired tumor blood vessels can deliver oxygen and drugs to tumor cells more efficiently, increasing the sensitivity to chemotherapy. Antiangiogenic therapy can produce a specific “time window”, a break when structure and function of tumor blood vessels and
Although the theory of normalization of tumor vessels has been widely accepted and confirmed by experiments, it may not be the only underlining mechanism. Changes in the tumor microenvironment after antiangiogenic therapy are so complex that the normalization of tumor blood vessels may not be the only early response. Aniaux et al.[18,19] found that treatment with SU5416 and Vandetanib increased oxygen in tumor tissues; however, this effect was the result of decrease of oxygen consumption which was not related to the remodeling of tumor vessels and tumor perfusion. Further studies revealed that SU5416 increased oxygen content in tumor tissues by inhibiting mitochondrial respiratory function.

Challenges and prospects

Although tremendous development on the combination therapy of antiangiogenesis and radiation are underway, there are many problems to be solved. As the regulation of tumor angiogenesis involves many vascular factors whose mechanisms are extremely complex, single-target antiangiogenic therapy may result of drug resistance. In such scenarios multitarget drugs would be the direction of future research. The combination therapy of antiangiogenesis and radiation relies on a comprehensive understanding of antitumor mechanism. In addition, the joint clinical research is immature. There are many practical issues to overcome, such as the best time and dose of drug administration of combination therapy and the lack of effective means to monitor treatment response. Therefore, the comprehensive knowledge of the mechanism of the combination therapy, tumor types and populations, the optimal dose and time, therapeutic order, efficacy and toxicity prediction will serve as the theoretic guidelines for the development of more rational combination cancer therapy.

Prevalence of tumor hypoxia seriously affects radiation therapy. Antiangiogenic therapy represents a new way to overcome the negative effects of tumor hypoxia. Certain progress has been achieved in the clinical antiangiogenic therapy in combination with chemotherapy. Antiangiogenic therapy combined with radiation therapy is one of the hot topics in radiation oncology and many clinical trials are underway.

References


