Review

Exploiting Tumor Hypoxia in the Treatment of Solid Tumors

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Abstract : Studies have shown that reduced oxygen tension (hypoxia) in solid tumors adversely affects the outcome of radiotherapy and chemotherapy. Besides being an independent prognostic marker of poor treatment outcome, hypoxia represents a physiological difference that can be utilized for selective cancer treatment. Since severe hypoxia does not occur in normal tissue, it may be exploited for therapeutic gain. One strategy is to use drugs that are toxic only under hypoxic conditions, and the first drug of this class to enter clinical testing, tirapazamine, is showing considerable promise. The second way to exploit hypoxia is to take advantage of the selective induction of the transcription factor hypoxia-inducible factor 1 (HIF-1) under hypoxic conditions. Gene therapy strategies based on this concept are in development.

Key Words : tumor hypoxia, metastasis, mutation, tirapazamine, gene therapy

Introduction

Solid tumors make up more than 90 % of all human cancers. They grow from a single, mutated normal cell and lead to significant morbidity and mortality either from growth of the tumor into surrounding normal tissue (as in the case of brain tumors) or, for most cancers, by metastasizing to vital organs throughout the body such as the lungs and liver. To grow beyond a small focus of tumor cells, however, the newly developing cancer, whether it is the original tumor or a metastasis, has to develop its own blood supply, which it does by stimulating the growth of cells from surrounding vessels into the tumor, a process known as angiogenesis. However, these newly formed blood vessels in the tumor are very different from normal vasculature. They are typically highly irregular and tortuous, have arterio-venous shunts and blind ends, lack smooth muscle or nerves, have incomplete endothelial linings and basement membranes and increase vascular permeability. As a result, blood flow is often sluggish and highly irregular, making the delivery of oxygen and nutrients to the tumor cells much less efficient than it is in normal tissues. Consequently, this leads to low overall levels of oxygen in most tumors, with

many areas extremely hypoxic. This occurs because tumor cells are further from blood vessels than the normal diffusion distance of oxygen (about 100 μm), the so-called chronically hypoxic cells, and because temporary stopping of blood flow through a particular vessel generates acutely hypoxic cells.

Studies using oxygen electrode measurements have demonstrated low oxygen levels in a variety of human solid tumors, including those of the brain, head and neck, breast, cervix and soft tissue sarcomas. While in normal tissues polarographic electrode measurements of oxygen partial pressure ($p\text{O}_2$) are in the 24–66 mmHg range, the pre-therapeutic oxygenation status of human malignancies presents median $p\text{O}_2$ readings of 2 mmHg (cervical carcinoma) to 28 mmHg (breast carcinoma), with fractions of measurements below 2.5 mmHg ranging from 5 % (soft tissue sarcoma) up to 82 % (FIGO 111 cervical carcinoma) ¹⁾.

Problems of hypoxia in solid tumors

It has been known for many years that well-oxygenated cells and tissues are more sensitive to the killing effects of ionizing radiation than those under hypoxic conditions. This is because oxygen molecules react rapidly with the free-radical damage produced by ionizing radiation in DNA, thereby ‘fixing’, or making permanent, the DNA damage that leads to cell death. The resistance of hypoxic cells to killing by radiation stimulated a number of clinical trials designed to overcome tumor hypoxia by having patients breathe 100 % oxygen at increased pressures. These trials have shown only marginal benefit, probably because breathing-increased levels of oxygen would not be expected to overcome acute hypoxia. Nevertheless, a number of studies using oxygen electrodes have demonstrated that the more hypoxic tumors are the ones that respond poorly to radiotherapy and have much lower cure rates ²⁾.

Although similar studies to those correlating radiotherapy outcome with oxygen electrode measurements have not been performed with chemotherapy, there is considerable evidence, from experimental studies with animal tumors, that hypoxic cells are also resistant to most anticancer drugs. However, this is not generally because cells low in oxygen are intrinsically resistant to most anticancer drugs; it is because hypoxic cells must be those furthest from functioning blood vessels, and it is also because cells at low oxygen levels divide much less rapidly than those that are fully oxygenated. These two factors lead to resistance to anticancer drugs; first, because chemotherapy drugs have to reach tumor cells from the blood vessels; and second, because the majority of anticancer drugs are only effective against rapidly proliferating cells ³⁾.

Hypoxia increases malignant progression and metastasis

Some clinical studies have shown that low oxygen levels in tumors are associated with increased metastasis; this has been shown for carcinoma of the cervix ⁴⁾ and soft tissue sarcomas ⁵⁾. Experimental studies suggested three possibilities. First, in experiments with cells in vitro and with transplanted tumors, hypoxia both in vitro and in vivo was shown to lead to cell death that depends on wild-type p53 ⁶⁾. Cells with mutated p53 are not susceptible to hypoxia-induced apoptosis. This means that hypoxia in tumors is a strong selection pressure for the development of mutated p53, an event that will increase the likelihood of an increasingly malignant phenotype. Second, it has also been shown experimentally that hypoxia can lead to increased mutations ⁷⁾. Finally, hypoxia is also a major stimulus for the increased expression of a large number of genes relevant to the growth and survival of cancer cells:

these genes include those encoding the angiogenic agent vascular endothelial growth factor (VEGF), the erythrocyte producing factor erythropoietin (EPO), glycolytic enzymes and signalling molecules⁸⁾. Therefore, it is still a matter of speculation whether hypoxia is a selection pressure for a more malignant phenotype, increased mutations, or enhanced expression of the prometastatic genes that are chiefly responsible for the increased metastasis of hypoxic tumors.

Drugs exploiting tumor hypoxia

Two ways by which tumor hypoxia could be an important therapeutic target are suggested. First, it is a major difference between tumors and normal tissue - thus, a drug that is cytotoxic only to hypoxic cells would be selectively toxic to tumor cells. Second, within the tumor, it is the hypoxic cells that are resistant to standard therapy. Thus, an agent selectively toxic to hypoxic cells would overcome the resistance to standard therapy of the solid tumor as a whole.

Mitomycin C, a quinone antibiotic that requires reductive metabolism for activity, is the prototype anticancer drug of this class. Introduced into clinical use in 1958, mitomycin C has demonstrated activity towards a number of different tumors in combination with other chemotherapeutic drugs and radiation. Sartorelli and colleagues suggested that what they thought would be the lower oxidation-reduction (redox) potential of tumor relative to normal tissue might be exploited to obtain greater activation of this compound through its cytotoxic derivatives⁹⁾. Although tumor redox potential did not turn out to be key for the activity of mitomycin C, Sartorelli and Rockwell were able to show that this drug preferentially kills hypoxic cells *in vitro*¹⁰⁾. However, the differential toxicity is modest: the ratio of drug concentrations under normoxic to hypoxic conditions for the same level of cell kill (hypoxic cytotoxicity ratio, HCR) is in the range of 1 (no differential effect) to 5¹¹⁾. In addition, extremely low levels of oxygen are required to obtain maximum cytotoxicity¹¹⁾. Nonetheless, this can be sufficient to overcome the resistance of hypoxic cells in animal tumors, and clinical trials have reported higher cure rates for head and neck cancers on adding mitomycin C to radiotherapy than with radiotherapy alone¹²⁾. As mitomycin C is a standard chemotherapy agent with systemic toxicity towards well-oxygenated cells, it is not clear whether the improved cure rates over radiotherapy alone were the result of selective killing of hypoxic cells. However, the results of the clinical trials are promising.

Some time ago, Brown and colleagues discovered a drug with a much higher selective toxicity for hypoxic cells, tirapazamine (TPZ)¹³⁾. The HCR of TPZ is around 300 for the SCC VII tumor cell line (Fig.1). They and other investigators have tested many different cell lines of human and rodent origin and have shown that hypoxic cells are uniformly much more sensitive to cell kill by TPZ than are well-oxygenated cells, with HCR values typically in the range of 50-150.

The mechanism for this preferential toxicity to hypoxic cells is shown in Fig.2. TPZ is a substrate for various intracellular reductase enzymes, which can add a single electron to the molecule, thereby producing a free-radical intermediate. In the presence of oxygen, this free radical is rapidly oxidized back to the parent molecule with the formation of a superoxide radical. However, in the absence of oxygen this does not occur, and the highly reactive TPZ radical will remove hydrogen atoms from nearby macromolecules, causing them structural damage. If this nearby molecule is DNA, then the TPZ radical produces both single- and double- stranded breaks leading to chromosome aberrations and cell death

Fig. 1. Killing of hypoxic and aerobic mouse SCC VII tumor cells assayed by clonogenic survival after 1 hour exposure of the cells to TPZ under either hypoxic or aerobic conditions *in vitro*²⁾. The HCR is the ratio of drug concentrations under aerobic-hypoxic conditions to produce equal cell kill; in this cell line, the HCR is approximately 300.

which are similar to those caused by high LET radiation as responsible for the toxicity under hypoxia¹⁴⁾. It was also revealed that metabolism by a reductase, or reductases, associated with the nuclear matrix might lead to the cytotoxic effects of TPZ. It appears that, although there are several different reductases that metabolize TPZ both in the nucleus and in the cytoplasm, the reactivity of the TPZ radical is so high that only those radicals produced very close to DNA—such as those produced close to the nuclear matrix—can damage the DNA to form the lethal double-stranded breaks¹⁵⁾.

As TPZ is selectively toxic to hypoxic cells, it would not be expected to kill all the cells in the tumor because many of those cells are well oxygenated. This has been found to be the case both in experimental animals and in clinical trials—TPZ alone has no effect on overall tumor growth. However, when combined with radiotherapy-type regimens to treat experimental tumors, TPZ is highly effective at potentiating tumor cell kill¹⁶⁾. It is also very effective at potentiating the anticancer activity of the chemotherapeutic drug cisplatin¹⁷⁾, an interaction that occurs at the cellular level and depends on hypoxia¹⁸⁾. Following favorable results in Phase I and II studies with the combination of cisplatin and TPZ, a Phase III, multi-institutional randomized clinical trial was performed with TPZ combined with cisplatin in patients with advanced non-small cell lung cancer. The results show that TPZ combined with cisplatin resulted in a doubling of the overall response compared with treatment with cisplatin alone, and was responsible for a significant increase in the median survival time of the patients¹⁹⁾. This

Fig. 2. The mechanism of the preferential hypoxic cytotoxicity of TPZ. Under both aerobic and hypoxic conditions, the drug is reduced by an intracellular reductase to form a highly reactive radical, which can cause DNA single- and double-stranded breaks. In the presence of oxygen, oxygen will take the free electron to itself, thereby back-oxidizing the radical to the inactive parent drug.

increase in antitumor activity occurred without any evidence of increased systemic toxicity of the anticancer drug cisplatin as was also seen in experimental animal systems¹⁷⁾.

Meanwhile, we have elucidated various characteristics of TPZ using a method for analyzing the responses of quiescent cells in solid tumors²⁰⁾. TPZ is very effective in the control of intratumor quiescent cells which are radio- and chemo-resistant due to their much higher hypoxic fractions than proliferating cells, its toxicity is enhanced by combination with mild temperature hyperthermia (40°C, 60 min)²¹⁾, and the use of TPZ in the treatment of solid tumors causes a shift from the proliferating to quiescent state *in vivo*²²⁾. TPZ reduces the repair capacity of both proliferating and quiescent tumor cells more than γ -rays or cisplatin²³⁾, which is compatible with the above-mentioned mechanism that active TPZ radicals are produced at high local concentrations by activating enzymes close to DNA.

Another hypoxic-selective drug that shows promise is AQ4N, a di-N-oxide analogue of mitoxantrone. Following reductive activation, AQ4N is converted to AQ4, which has a high affinity for DNA and inhibits the essential enzyme topoisomerase II²⁴⁾. Topoisomerase II is an essential nuclear enzyme whose major function is to regulate the topological state of DNA during replication and chromosome

condensation and segregation. It does this by catalyzing the transient cleavage of one DNA double helix, passage of an intact DNA strand through the break, and resealing of the broken DNA strand. It is also the key cellular target for a number of clinically important anticancer drugs including etoposide and the anthracyclines doxorubicin, daunorubicin and m-AMSA. Unlike other bioreductive agents, AQ4N has the advantage that the reduced product remains active even if the hypoxia that led to bioactivation is transient, or if the active compound diffuses away from the hypoxic regions. Indeed, the drug has shown considerable activity when combined with radiation and anticancer drugs ²⁵⁾, and will soon enter Phase I clinical trials.

Exploiting tumor hypoxia in gene therapy

Hypoxia-targeted gene therapy is the latest approach that aims to exploit this unique physiological feature of solid tumors, with the major goal to eradicate therapy-resistant malignant populations, while sparing normal tissue from damage. The major obstacle facing efficient gene therapy of tumors is the development of a tumor-specific delivery system. Accordingly, the protocols in cancer gene therapy now involve local administration - usually by needle injection - of the vectors directly into the tumors. Although this might provide a proof of concept, it has limited applicability to cancer in general because it is the metastases that are often undetected, too numerous, or inaccessible to direct injection. However, one alternative to direct targeting of tumors would be to have the therapeutic gene transcribed or translated by some tumor-specific property, so that, even if the vector were distributed in all tissues, expression of a particular protein would occur specifically in the tumors.

One strategy to exploit hypoxia in solid tumors would be to develop a promoter that is highly responsive to hypoxia-inducible transcription factor 1 (HIF-1) that would drive the expression of a therapeutic gene specifically in the tumor ⁸⁾. HIF-1 is a transcription factor expressed specifically under hypoxic conditions. It is composed of two proteins: HIF-1 α , which increases under hypoxic conditions, and HIF-1 β , which is expressed in both normoxic and hypoxic cells and had previously been identified as the arylhydrocarbon receptor nuclear translocator (ARNT). Expression of an enzyme not normally found in the human body could, under the control of a hypoxia-responsive promoter, convert a nontoxic pro-drug into a toxic drug in the tumor (Fig.3). Proof of this concept has been obtained using a number of different enzymes, such as the bacterial cytosine deaminase, which converts the nontoxic 5-fluorocytosine to the anticancer drug 5-fluorouracil, and significant anticancer efficacy has been obtained with tumors expressing this protein using a constitutively active promoter ²⁶⁾.

Prospects

The much lower oxygen levels of cells in solid tumors compared with those in normal tissue provide an opportunity for selective anticancer therapy, but many questions remain to be answered.

First, we need to know the extent to which tumor hypoxia contributes to tumor resistance to commonly used anticancer drugs. It would be of great value in selecting the appropriate treatment if we knew the extent to which hypoxic tumors were resistant to specific anticancer drugs.

Second, we must investigate the mechanism by which the tumor microenvironment, especially hypoxia, causes increased metastatic spread. As mentioned above, the possibilities include selection of

Fig. 3. How the hypoxic environment of solid tumors might be used in gene therapy to produce tumor-specific expression of an enzyme that can metabolize a nontoxic pro-drug to a toxic drug specifically in the tumor.

a more malignant phenotype, an increased mutation rate, and the induction of genes that could confer a pro-angiogenic, pro-metastatic phenotype. Characterization of these genes could be important not only to understand the mechanism of tumor spread but also to provide prognostic information for a particular cancer patient.

Third, what is the mechanistic basis of some of the unique side effects of TPZ, such as muscle cramp? It has been speculated that this is the result of the generation of superoxide radicals under normoxic conditions, but this has yet to be proven.

Another major outstanding question is the identity of the matrix-associated reductase that activates TPZ to form its DNA-damaging radical. Identification of this enzyme would provide a guide as to the likely activity of TPZ in the tumors of individual patients.

Finally, is HIF-1 induction necessary for the continued growth and survival of tumors? Results demonstrating the importance of this transcription factor suggest that it could be a novel, tumor-specific target for anticancer therapy.

Conclusion: Turning hypoxia from a problem to an advantage

Although the unique presence of hypoxia in human tumors is a negative prognostic indicator for treatment efficacy, it also provides an important target for selective cancer therapy. Such selective, hypoxia-based therapy has yet to be put into general clinical practice, but a number of promising strategies are currently under investigation both in the laboratory and in the clinic.

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固形腫瘍の治療における腫瘍低酸素の活用

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要 旨：従来の研究は、腫瘍内の低酸素が放射線治療および化学療法治療の成績を低下させることを明らかにしてきた。治療成績を低下させる指標であるにもかかわらず、低酸素は選択的な癌治療の実現のために利用され得る生理学的な相違点であることも示している。正常組織では強度の低酸素状態は生じにくく、低酸素を治療効果比向上のための一つの標的として利用することができる。一つの戦略は、低酸素状態のみで毒性を有する薬剤を利用することであり、臨床試行に至った最初の薬剤としてチラパザミンがあり、現在有望視されている。低酸素状態を利用する第2の方法は、低酸素状態における hypoxia-inducible factor 1 (HIF-1) 転写の選択的誘導を活用することであり、この考え方に基づいた遺伝子治療の開発も現在進行中である。