The Unique Physiology of Solid Tumors: Opportunities (and Problems) for Cancer Therapy

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Abstract

The physiology of solid tumors differs from that of normal tissues in a number of important aspects, the majority of which stem from differences between the two vasculatures. Compared with the regular, ordered vasculature of normal tissues, blood vessels in tumors are often highly abnormal, distended capillaries with leaky walls and sluggish flow. Tumor growth also requires continuous new vessel growth, or angiogenesis. These physiological differences can be problems for cancer treatment; for example, hypoxia in solid tumors leads to resistance to radiotherapy and to some anticancer drugs. However, these differences can also be exploited for selective cancer treatment. Here we review four such areas that are under active investigation: (a) hypoxia-selective cytotoxins take advantage of the unique low oxygen tension in the majority of human solid tumors. Thirapazamine, a drug in the final stages of clinical trials, is one of the more promising of these agents; (b) leaky tumor blood vessels can be exploited using liposomes that have been sterically stabilized to have a long intravascular half-life, allowing them to selectively accumulate in solid tumors; (c) the tumor microenvironment is a stimulus to angiogenesis, and inhibition of angiogenesis can be a powerful anticancer therapy not susceptible to acquired drug resistance; and (d) we discuss attempts to use gene therapy activated either by the low oxygen environment or by necrotic regions of tumors.

Introduction

Tumor Physiology: A Relatively Unexplored Target for Cancer Therapy. Nonsurgical methods of cancer treatment, primarily radiation therapy and chemotherapy, rely almost exclusively on agents that kill cells. The main problem with these current treatments, however, is that they do not, in general, have specificity for cancer cells. In the case of radiation therapy, a degree of specificity is achieved by localizing the radiation to the tumor and its immediate surrounding normal tissue. For anticancer drugs, it is primarily the rapid proliferation of many of the cancer cells that makes them more sensitive to cell killing than their normal cellular counterparts. However, both modalities are limited by their cytotoxic effects on normal cells. In the case of radiotherapy, normal tissue surrounding the tumor limits the radiation dose, whereas for anticancer drugs, it is usually the killing of rapidly dividing normal cells, such as those in the bone marrow, hair follicles, and epithelial cells lining the gastrointestinal tract, that limit the dose that can be given.

To achieve greater efficacy with present day treatments, investigators are attempting to exploit differences between normal and malignant cells at the cellular and molecular level. However, there is a second critical difference between normal and malignant tissues that has the potential for exploitation to produce more specific anticancer therapy. As this review will detail, the physiology of solid tumors at the microenvironmental level is sufficiently different from that of the normal tissues from which they arise to provide a unique and selective target for cancer treatment. To date, targeting tumor physiology for anticancer therapy has received considerably less attention than approaches based on the cellular and molecular differences between transformed and untransformed cells. Table 1 lists the principal differences in physiology between normal and malignant tissues that can be exploited (and can also be a problem) in cancer treatment.

The Vasculature: Basis for the Unique Physiology of Solid Tumors. The underlying differences between the physiology of normal and tumor tissues stems from the tumor vasculature. This is composed of two types of vessels: the existing vessels in normal tissues into which the tumor has invaded; and tumor microvessels arising from neovascularization resulting from increased expression of proangiogenic factors produced by tumor cells. Both types of vessels develop structural and physiological abnormalities that have become a hallmark of the tumor microvasculature. Although early studies of tumor blood flow described marked heterogeneity and often sluggish flow (1), it was the later studies of vascular casting techniques and window chamber preparations that identified the structural basis for these flow inhomogeneities (2-4). These studies showed that tumor blood vessels are highly irregular, tortuous, have arterio-venous shunts, blind ends, lack smooth muscle or enervation, and have incomplete endothelial linings and basement membranes (Fig. 1). As a result, blood flow is often sluggish, highly irregular, and the vessels much "leakier" than those in normal tissues. These characteristics of tumor vasculature lead to the physiological differences described in the following sections that present both problems and opportunities for treatment of solid tumors.

Hypoxia-selective Cytotoxins

Tumor Hypoxia. The pioneering work of Gray et al. (5) demonstrated that the sensitivity to radiation damage of cells and tissues depended on the presence of oxygen at the time of irradiation. The histological studies of human lung adenocarcinomas by Thomlinson and Grey (6) provided a mechanism by which cells could be hypoxic in tumors. They postulated that because of their unrestrained growth, tumor cells would be forced away from vessels beyond the effective diffusion distance of oxygen in respiring tissue, thereby becoming hypoxic and eventually necrotic. Given typical values for intracapillary oxygen tensions and oxygen consumption rates, the oxygen diffusion distance would be approximately 150 μm (Fig. 2).

Fig. 2 shows two important further consequences of reducing oxygen concentration: (a) the fraction of proliferating cells and/or the rate of cell proliferation decreases as a function of distance from the vasculature (7, 8), a phenomenon that, at least in vitro, is largely, or wholly, the result of decreasing oxygen levels (9). An important consequence of this hypoxia-induced inhibition of proliferation is that because most anticancer drugs are primarily effective against rapidly dividing cells, their effectiveness would be expected to fall off as a
function of distance from blood vessels. This has been shown experimentally (10); and (b) because hypoxic cells must be the ones most distant from blood vessels, they will be exposed to lower concentrations of drug than those adjacent to blood vessels, primarily as a result of the metabolism of such agents through successive cellular layers.

Thus, tumor hypoxia would be expected to be an important factor leading to resistance to radiotherapy (because of hypoxia, per se, affecting cellular radiation sensitivity) and to chemotherapy (because of lower proliferation and lower drug concentrations in the hypoxic cells). The consequent reduction of cell kill to anticancer treatment as a function of distance from tumor blood vessels is shown in Fig. 2.

What is the evidence for this model? Hypoxia is a common feature of both human and animal tumors (11, 12). The vast majority of human solid cancers have median pO2 levels lower than their normal tissue of origin (12, 13). In animal tumors, it can be shown that these hypoxic cells are also viable and contribute to the resistance of transplanted tumors to both radiation (14) and to some anticancer drugs (15, 16). In human tumors, there is direct evidence from measurements of oxygen levels that hypoxia contributes to resistance to radiotherapy (17–19). Similar studies have not been performed with chemotherapy, although the evidence of a strong correlation between the response of head and neck cancers to chemotherapy and to radiotherapy implicates hypoxia as a cause of drug resistance (20, 21).

Hypoxia in solid tumors, however, has an important consequence in addition to conferring a direct resistance to radiation and chemother-apy. Graeber et al. (22) showed recently that low oxygen levels caused apoptosis in minimally transformed mouse embryo fibroblasts and that this apoptosis depended to a large extent on wild-type p53. They further showed, using these same cells growing as solid tumors in immune-deprived mice, that apoptosis colocalized with hypoxic regions in tumors derived from p53 wild-type mice. In tumors derived from p53 −/− cells, there was much less apoptosis and no colocalization with tumor hypoxia. These findings provide evidence that hypoxia, by selecting for mutant p53, might predispose tumors to a more malignant phenotype. Clinical data support this conclusion. Studies both with soft tissue sarcomas (23) and with carcinoma of the cervix (24) have shown that hypoxic tumors are more likely to be metastatic.

The model shown in Fig. 2 of hypoxic cells occurring at the diffusion distance of oxygen is the classic model of tumor hypoxia generally attributed to Thomlinson and Gray (6). However, we and others have proposed that tumor hypoxia can occur in a second way, by temporary obstruction or cessation of tumor blood flow—the so-called acute hypoxia model (25, 26). Definitive evidence for this type of acute hypoxia arising from fluctuating blood flow has come from elegant studies with transplanted tumors in mice using diffusion-limited fluorescent dyes (27, 28). Because fluctuating blood flow has also been demonstrated in human tumors (29, 30), it is likely that this type of hypoxia is also present in human tumors. The consequences of acute hypoxia will be similar to those of the diffusion-limited hypoxia.
Any cells surrounding a closed blood vessel will be resistant to radiation killing because of their lack of oxygen at the time of radiation and will be exposed to lower levels of anticancer drugs than those surrounding blood vessels with normal flow. This would be expected to lead to inhomogeneities in response to anticancer agents, as has been observed in experimental tumors (31).

**Hypoxia-selective Cytotoxins.** Can the low oxygen levels in tumors be turned from a disadvantage to an advantage in cancer treatment? Such a possibility was proposed over 20 years ago by Lin et al. (32), who reasoned that compounds based on the quinone structure of mitomycin C might be more active in hypoxic tumors. It was known at that time that mitomycin C required metabolic reduction of the benzoquinone ring to produce the cytotoxic bifunctional alkylating agent. Lin et al. (32) reasoned that a lower oxidation reduction (redox) potential for tumor tissue relative to most normal tissues could increase reductive activation of these quinone derivatives in tumors. Although this was not the correct mechanism for the increased cytotoxicity of mitomycin C and certain analogues toward hypoxic cells (much lower levels of hypoxia are needed to change cellular redox potential), these studies were important in suggesting the potential of hypoxia-activated drugs and led to the concept of selectively killing the hypoxic cells in solid tumors (33–37).

It is important to note that specifically killing the hypoxic cells in tumors has greater therapeutic potential than oxygenating the cells or chemically sensitizing them to radiation or chemotherapy. Not only is the killing tumor specific (hypoxia is tumor specific), but the cells killed are the ones resistant to conventional therapy. This principle of “complementary cytotoxicity” is illustrated in Fig. 2. The combined killing of two agents with complementary cytotoxicity is potentially much greater than that of two agents acting on the same cell population. The other major advantage of hypoxia-selective cytotoxins is their potential for providing enhancement to the killing of standard anticancer drugs. This cannot be done by temporarily oxygenating the hypoxic tumor cells.

There are presently three different classes of hypoxia-specific cytotoxins that are in use clinically or are being developed for clinical use. They are the quinone antibiotics, the nitroimidazoles, and the benzotiazine di-N-oxides. In the quinone class, the three principal agents of current clinical interest are mitomycin C, porfiromycin, and E09. All are structurally similar and require reductive metabolism for activity. Each is converted on reductive metabolism to a bifunctional alkylating agent and probably produce their major cytotoxic activity through the formation of DNA interstrand cross-links. Reviews of the mechanism of action, pharmacology, and preclinical and clinical activities of these drugs have been published (38–40).

Mitomycin C, justifiably considered to be the prototype bioreductive drug (37), was introduced into the clinic in 1958 and has demonstrated efficacy toward a number of different tumors in combination with other chemotherapy drugs and with radiation. However, its selective toxicity toward hypoxic cells is modest, with values for hypoxic cytotoxicity ratios (the ratio of drug concentration to produce equal cell kill for aerobic and hypoxic cells) of 1 (no preferential toxicity) to approximately 5 (37, 41–43). However, based on this activity, mitomycin C has been combined with radiotherapy in two randomized trials of head and neck cancer (44, 45), the pooled results of which gave a statistically significant disease-free survival benefit. Whether this promising finding is the result of preferential cytotoxicity of mitomycin C toward hypoxic cells or to cytotoxicity to both aerobic and hypoxic cells is, however, open to debate (40). Nonetheless, encouraged by this success, the Yale group is now testing porfiromycin, a drug that has a somewhat greater hypoxic-selective toxicity than mitomycin C.

The third drug in this series, E09, is a much more efficient substrate for DT-diaphorase than either mitomycin C or porfiromycin and shows high toxicity to both aerobic and hypoxic cells in cells with high DT-diaphorase levels. Cells with low DT-diaphorase levels are much less susceptible to killing by E09 under aerobic conditions but show a high (up to 50-fold) preferential toxicity toward hypoxic cells. However, the pharmacokinetics of this agent work against its clinical utility, and Phase I clinical studies have shown little activity of this drug (46).

A second class of bioreductive agents is the nitroimidazoles, the first two of which, metronidazole and misonidazole, were extensively tested as hypoxic radiosensitizing agents (47). Further drug development by Adams et al. (48) produced a compound, RSU1069 (1(2-nitro-1-imidazolyl)-3-(1-aziridinyl)-2-propanol), which has been shown to be a highly efficient cytotoxic agent with activity both in vitro and in vivo (48, 49). RSU1069 has a hypoxic cytotoxicity ratio of some 10–100 for different cell lines in vitro, and it, or its prodrg, RB6145, has shown excellent activity with mouse tumor models when combined with irradiation or agents that induce hypoxia (50). Unfortunately, however, clinical testing of RB6145 has been aborted due to irreversible cytotoxicity toward retinal cells (51).

TPZ is the first, and thus far, only representative of a third class of hypoxia-selective cytotoxins. This drug has a high selective hypoxic cytotoxicity (20–300 for different cell lines) and maintains its differ-

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The abbreviations used are: TPZ, tirapazamine; RES, reticular endothelial system; VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor; HRE, hypoxia-responsive element; 5-FC, 5-fluorocytosine.
to chromosome breaks as the principal mechanism of cell killing.

Oxygen can remove the additional electron from the TPZ molecule(s) leading to DNA damage, which is located in the cell nucleus, under hypoxic conditions (54). Recent work has shown that the activating enzyme(s) leading to DNA damage is shown.

Fig. 3. A schematic showing the metabolism of TPZ to its active free radical moiety. The toxic species has been identified as the radical formed by the 1-electron reduction of TPZ, with DNA double-strand breaks, leading to chromosome breaks as the principal mechanism of cell killing under hypoxia (54).

The mechanism of the selective toxicity of TPZ is illustrated in Fig. 3. The toxic species has been identified as the radical formed by the 1-electron reduction of TPZ, with DNA double-strand breaks, leading to chromosome breaks as the principal mechanism of cell killing under hypoxia (54).

Liposome Delivery

Tumor vessels are often leaky, demonstrating increased permeability to circulating large molecules, even up to sizes of 400 nm in diameter (65–67). This leakiness of the vasculature results from an incomplete or missing endothelial cell lining and basement membrane (65, 68). Increased permeability of tumor vasculature has allowed specific targeting of anticancer drugs to tumors using small liposomes of ~100-nm diameter that have been specially modified to remain in the circulation for long periods of time.

The discovery that drugs could be encapsulated in fatty vesicles or liposomes in the 1960s led to high enthusiasm that this system would fill the role of the “magic bullet” for drug delivery. However, animal experiments soon proved these early expectations to be misguided, because liposomes, when injected i.v., are rapidly recognized and removed by the RES. An elegant solution to this problem was found by coating lipids with carbohydrate groups to have them resemble erythrocytes (69–71). These developments gave rise to today’s sterically stabilized, or Stealth™, liposome which is coated with polyethylene glycol to provide a steric barrier to recognition and destruction by the RES (72). Such liposomes with encapsulated epirubicin or doxorubicin exhibit improved antitumor efficacy compared with conventional liposomes or to free drug (73). Stealth liposomes remain in the blood circulation for extended periods of time, with most of the liposomes remaining in the blood and only 10–15% ending up in the liver, a major improvement over conventional liposomes, the majority of which are rapidly captured by the RES in the liver (74).

Despite the impressive preclinical activity of both doxorubicin and vincristine encapsulated in sterically stabilized (Stealth) liposomes, none of the studies to date have attempted to determine the extent to which the increased antitumor activity is the result of increased tumor concentrations of liposomes carrying the drug or to prolonged exposure time of the tumor cells to drug. Presumably, both are important, although the benefit of prolonged exposure time is very likely to be dependent on tumor proliferation rate and the drug in question.

Clinical studies of doxorubicin encapsulated in Stealth liposomes (Doxil) began a number of years ago, and the results to date confirm the prolonged circulation time (plasma t1/2 of 45 h compared with ~10 h for free doxorubicin) and increased concentrations of free drug in pleural effusion of a variety of tumors in those patients treated with doxil compared with free doxorubicin (75). These Phase I/II studies have also identified a novel toxicity of doxorubicin in Stealth liposomes, i.e., a prolonged desquamating painful dermatitis primarily affecting hands and feet, possibly due to liposome-mediated deposition of drug in the skin. However, the results to date for a variety of solid tumors support the preclinical findings that increased antitumor efficacy will be likely with doxorubicin encapsulated in Stealth liposomes versus free drug.

Despite the obvious advantage provided by leaky blood vessels allowing high tumor concentrations of Stealth liposomes, it has to be recognized that the distribution of the liposomes is highly heterogeneous and concentrated in the perivascular interstitial regions (67, 76). Presumably, the antitumor activity of these heterogeneous deposits is the result of a slow release of drug throughout the tumor from these hot spots of high drug concentrations.
Antiangiogenesis

In the early 1970s, Folkman (77, 78) introduced what was then the controversial hypothesis that the growth of solid tumors was absolutely dependent upon new blood vessel formation, or angiogenesis, developing from outside the growing tumor mass. He also suggested that, this being the case, therapy aimed specifically at the angiogenic developing from outside the growing tumor mass. He also suggested controversial hypothesis that the growth of solid tumors was absolutely inhibit the growth of s.c. tumors, and to prevent metastatic spread have been isolated and shown to inhibit angiogenesis (82), to mark cells with viruses expressing a dominant negative mutant form of Flk-1 suppressed glioblastoma tumor growth. Also, the tumors that infection of the vasculature surrounding implanted glioblastomas, VEGF message was highly expressed adjacent to areas of focal necrosis (where hypoxia would be expected) and that new capillary bundles were localized alongside VEGF-producing cells. Further evidence that tumor hypoxia is an important signal for angiogenesis, and a determinant of tumor growth, is provided by the recent work of Maxwell et al. (94), who showed that tumors growing from cells deficient in the hypoxia-activated transcription factor, hypoxia-inducible factor 1, did not show increased VEGF induction adjacent to necrosis, were poorly vascularized, and grew much slower than tumors with competent hypoxia-inducible factor 1.

Because VEGF can be induced in normal cells by hypoxia (95, 96), the question arises as to the relative contributions to tumor angiogenesis from the microenvironment (i.e., hypoxia) and from malignancy per se (97). Mazure et al. (98) have shown recently that it is probably both: oncogenic transformation (at least by certain oncogenes) and hypoxia act synergistically to induce VEGF. Therefore, the use of agents that inhibit oncogenic ras activity may act as antimumor agents in part by inhibiting signaling pathways in the angiogenic activation cascade.

Antiangiogenesis therapy has several important advantages over standard anticancer treatment: (a) it targets a process that, under most circumstances, is tumor specific and therefore likely to have little systemic toxicity; (b) it has the advantage that with an endothelial cell target, there is not the problem of the drug having to reach tumor cells, often many cell layers from the vasculature; and (c) one of the most important advantages of antiangiogenic therapy is that the genetic stability of endothelial cells (as opposed to tumor cells) should prevent the development of drug resistance on repeated administration of the agent. This was first proposed by Kerbel (99) and recently dramatically demonstrated by Boehm et al. (100), who induced multiple regressions in transplanted mouse tumors by repeated administration of endostatin. This concept is illustrated in Fig. 4.

In summary, antiangiogenic strategies are now showing a great deal of promise in murine tumor models. The responses of such transplanted tumors to inhibitors of VEGF or its receptor Flk-1, or to the natural antiangiogenic peptides, angiotatin and endostatin, are extremely impressive. We believe it would not be overly optimistic to conclude that if human spontaneous tumors are as dependent on continued angiogenesis as are the more rapidly growing transplanted tumors in rodents (and this is a major unknown), then these strategies could constitute a very important advance in cancer therapy.
EXPLOITING TUMOR PHYSIOLOGY

Fig. 4. The mechanism by which resistance to many standard anticancer drugs develops by outgrowth of drug-resistant tumor cell subpopulations, contrasted with the lack of development of resistant tumors when antiangiogenesis therapy is directed against endothelial cells [redrawn from Kerbel (112)].

Gene Therapy

Activated by Hypoxic Stress Response. The newest direction for exploiting tumor physiology is aimed toward the evolving field of gene therapy. In this novel approach to anticancer therapy, genetic material is transferred into cells by a variety of techniques with the ultimate goal of selective killing of cancer cells and the sparing of normal cells. At present, this selective killing of tumor cells can be achieved either by controlling vector delivery and activity or by transgene expression. Previous studies by Hallahan et al. (101) have tested the feasibility of intratumoral control of transgene expression using a radiation-inducible promoter ligated to the TNF-α gene. They were able to demonstrate radiation increased TNF-α production intratumorally after a 50-Gy single dose of ionizing radiation. Furthermore, the radiation-induced increase in TNF-α production resulted in increased tumor cell killing. These experiments on radiation-inducible gene expression establish the proof in principle that a strategy to control gene expression by stress-responsive promoters is feasible in vivo.

A direct test of the ability of the low oxygen conditions to selectively control gene expression and increase cell kill was performed by Dachs et al. (102), who linked a HRE from the mouse phosphoglycerate kinase-1 gene to the bacterial cytosine deaminase-encoding gene and stably transfected it into HT1080 cells. To test the hypothesis that only under low oxygen conditions would the expression of the bacterial cytosine deaminase gene be increased, the HT1080 cells that were stably transfected with the HRE-cytosine deaminase gene construct were exposed either to 5-fluorouracil or to 5-FC under aerobic and hypoxic conditions. Only hypoxic cells were sensitive to 5-FC, which inactive prodrug, which requires enzymatic conversion to 5-fluorouracil by cytosine deaminase to be cytotoxic. The increased sensitivity of HT1080 transfectants to 5-FC suggested that in vitro HREs could be used to control the expression of a prodrug activating enzyme. At present, we do not have functional data on the ability of HREs to control the activation of nontoxic prodrugs into a tumoricidal form in vivo. However, the ability of HREs to induce the activity of a reporter gene has been evaluated intratumorally, and HREs seem to be able to transcriptionally regulate gene expression under low oxygen conditions in vivo (102).

There are numerous implications of these studies for cancer gene therapy: (a) the use of HREs will provide a selective means of controlling gene transcription in a wide variety of solid tumors based on the lower oxygen levels of tumors compared with normal tissues; (b) the use of enzymes under the control of an HRE adds a safeguard (compared with expression of a toxic substance), because increased expression of the prodrug activating enzyme is itself nontoxic; (c) the expanding list of inactive cytotoxic prodrugs and prodrug activating enzymes increases the possibility of finding the most efficacious combinations for different tumor types; and (d) continued research on the regulation of gene induction by hypoxia should offer new transcriptional regulatory elements as well as transcriptional stimulating elements that will increase the dynamic range and specificity of transcriptional responses to a low oxygen environment. Potentially this approach, therefore, would allow the activation of a nontoxic prodrug to its toxic metabolite selectively in solid tumors. However, as with most forms of gene therapy, targeting the constructs to the tumor remains a major hurdle.

Targeted by Tumor Necrosis. It has been known for several decades that certain species of anaerobic bacteria of the genus Clostridium can selectively germinate and grow in the hypoxic/necrotic regions of solid tumors after i.v. injection of spores. This was first dramatically demonstrated by Malmgren and Flanagan (103) with C. tetani, the causative agent of tetanus. Mice, when injected i.v. with spores of this bacteria, remained healthy unless they had tumors, in which case death by tetanus resulted within 48 h. This was caused by germination of the bacteria in the tumors and release of toxins systemically. Mose et al. (104) later isolated a nonpathogenic strain of C. sporogenes (later renamed C. oncolyticum), which germinated in tumors after i.v. injection of the spores, causing tumor lysis and shrinkage of the tumors (104). Extensive preclinical testing was followed by clinical trials of this agent, particularly with patients with glioblastoma who received injections of up to $10^{10}$ C. oncolyticum spores (105, 106). Lysis was demonstrated in the tumors, with no evidence of clostridial germination or tissue destruction in the surrounding normal tissue. With the exception of mild to moderate fever, the patient suffered no ill effects from the injection of these organisms. However, no clinical benefit was demonstrated, presumably

thereby generating the prodrug-activating enzyme in the tumor, which can subsequently activate a prodrug into a toxic drug. Because the enzyme would only be expressed in the hypoxic/necrotic areas of the tumor, toxic drug will diffuse from the hypoxic/necrotic areas with the highest concentrations of drug in these areas. This would activate a prodrug to a toxic drug in these areas. Toxic drug will diffuse from the hypoxic/necrotic areas of the tumor, which can subsequently activate a prodrug to a toxic drug in these areas. Toxic drug will diffuse from the hypoxic/necrotic areas with the highest concentrations of drug in these areas. This would be expected to produce cytotoxicity complementary to that of conventional therapy in a manner similar to that shown in Fig. 2.

because of rapid regrowth of tumor from oxygenated tissue; therefore, clinical trials were discontinued.

We have proposed that these tumor-targeting clostridia could be genetically manipulated, thereby exploiting tumor hypoxia for a new form of gene therapy (107). Although it might be possible to obtain an antitumor effect with the bacteria expressing a toxic protein, we believe a more effective and potentially safer approach would be for the bacteria to express an enzyme that could convert a nontoxic produg into a toxic drug. Because the enzyme would only be expressed in the tumor, the conversion of a systemic produg to a toxic anticancer drug would occur only in the tumor (Fig. 5).

We have recently demonstrated the proof of this principle using genetically engineered C. beijerinckii, a species that also colonizes hypoxic areas in tumors (although not to the same extent of C. oncolyticum) (108). These clostridia were transformed with a plasmid expressing the E. coli enzyme, nitroreductase, which can convert the monofunctional alkylating agent, CB1954, into a potent bifunctional agent by rat (but not human) DT-diaphorase (111). We showed that both nitroreductase protein and activity could be detected in tumors from mice injected with clostridia carrying the nitroreductase gene but not in tumors derived from mice injected with control clostridia. Furthermore, no detectable nitroreductase protein was found in any of the normal tissues from mice expressing this protein in the tumors (108).

These studies establish the possibility of using tumor necrosis and/or hypoxia to target specific proteins to tumors and have these proteins expressed solely in the tumors. Future work is needed to achieve high enough stable expression of activating enzymes in the tumors to achieve antitumor activity by metabolism of produgs.

Conclusions

The vasculature and cellular environment, often termed the “microenvironment,” of solid tumors is different from that of normal tissues in several important respects. We have outlined in this review what we believe are the most important of these for cancer therapy. Several of these differences, e.g., high interstitial pressure and tumor hypoxia, have been recognized for some time as having detrimental effects on some types of cancer treatment. More recently, however, microenvironmental differences between normal and malignant tissues are being appreciated as opportunities for tumor-selective therapy. We review here four such opportunities that are in, or approaching, clinical testing: hypoxia-selective cytotoxins, sterically stabilized liposomes, antiangiogenesis, and gene therapy activated by hypoxia or by tumor necrosis. Each of these exploits a unique feature of solid tumors, thereby providing an exciting opportunity for tumor-selective cancer treatment.

References


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