Physiological Mechanisms of Treatment Resistance

15

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CONTENTS

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KEY POINTS

- The unique, considerably dynamic and thus complex physiology of tumors can markedly influence the therapeutic response to standard irradiation, chemotherapy, photodynamic therapy, endocrine therapy and immunotherapy.
- Acquired treatment resistance due to the impact of the hostile microenvironment adds to the "classical" drug resistance based on the molecular biology of tumors.
- The chaotic microvasculature leads to a significant impediment of delivery, an uneven distribution and a compromised penetration of drugs from tumor capillaries to more distant tumor cells.
- Interstitial transport of larger molecules (monoclonal antibodies, cytokines) by convection is inhibited.
- Low cell proliferation rates and cell cycle arrest distant from tumor microvessels can protect tumor cells from the effects of cytotoxic therapies whose activity is selective for rapidly dividing cell populations.
- Hypoxia directly and/or indirectly confers resistance to therapy. Direct effects are mediated through reduced generation of free radicals (some chemotherapy, photodynamic therapy) or lacking fixation of DNA damage (X- and γ-rays).
- Indirect hypoxia-driven effects are mostly based on changes in the transcriptome, in differential regulations of gene expression and in alterations of the proteome and genome.
- Anemia can lead to therapeutic resistance through deepening hypoxia and reducing the transport capacity of red blood cells for various antineoplastic drugs.

- Tumor acidosis is involved in acquired treatment resistance through a series of mechanisms including, inter alia, inhibition of cell proliferation, reduced cellular uptake and activation or an increased efflux of drugs.

Abstract

It is generally accepted that tumor perfusion, microcirculation, characteristics of the interstitial space of tumors, oxygen (and nutrient) supply, tissue pH distribution and the bioenergetic status—factors that are usually closely linked and that define the so-called pathophysiological microenvironment—can markedly influence the therapeutic response of malignant tumors to sparsely ionizing radiation, chemotherapy, photodynamic therapy, hormonal therapy and immunotherapy. Besides more direct mechanisms involved in the development of acquired therapeutic resistance, there are in addition, obstacles in intratumor pharmacokinetics of antitumor agents due to delivery problems caused by an inadequate and heterogeneous perfusion and barriers within the interstitial compartment. Indirect effects causing therapeutic resistance include lower cell proliferation rates and cell cycle arrest. Changes in transcriptome, alterations in gene expression and in the genome, genomic instability and clonal selection can drive subsequent events that are known to further increase resistance to therapy, in addition to critically affecting long-term prognosis.

15.1 Introduction

The physiology of solid tumors is uniquely different to that of normal tissues. It is characterized, inter alia, by a chaotic microvascular structure and function, O_2 depletion (hypoxia and anoxia, respectively), extracellular acidosis, significant interstitial fluid flow, and interstitial hypertension, creating a hostile pathophysiological microenvironment (see Chap. 4). This microenvironment is not static, but instead is quite dynamic (and therefore more complex than previously assumed), describing a situation that is not compatible with earlier, conventional dogmas.

Hypoxia and the other microenvironmental parameters are known to directly or indirectly confer resistance to non-surgical treatment modalities through limited access of therapeutics to the tumor, decreased radiosensitivity and drug action in the absence of O_2, critically reduced effects in tumor cells that are poorly proliferating and via changes in pH gradients, etc. Other mechanisms include the capacity of the hostile microenvironment to drive changes in gene expression, genomic instability and clonal selection.

15.2 Role of the Disorganized, Compromised Microcirculation as an Obstacle in Tumor Therapy

As already mentioned in Chap. 4, there is a disturbed balance of pro-angiogenic and anti-angiogenic molecules (yielding an unregulated angiogenesis), which leads to the development of a disorganized microvasculature and significant arterio-venous shunt perfusion and thus to an inefficient delivery of therapeutic molecules (e.g., drugs, cytokines and antibodies) and nutrients (e.g., oxygen and glucose) through the vascular system of the tumor (see Table 15.1). The situation is further aggravated by flow-dependent spatio-temporal heterogeneities in the distribution of plasma-borne drugs (and their metabolites).

The considerable impediment of fluctuating (intermittent) perfusion to successful cancer therapy has been comprehensively reviewed by Durand (2001) and Durand and Aquino-Parsons (2001 a,b).

The mean vascular density in most tumor areas is generally lower than that in normal tissues, and thus diffusion distances are enlarged. Penetration of drugs from tumor capillaries to tumor cells that are distant from them is therefore compromised. In these tumor regions distant to patent microvessels, some drugs (i.e., drugs with a short half-life within the circulation) cannot achieve sufficient concentrations to exert lethal toxicity for all of the viable cells further away from the tumor microvasculature system (Minchinton and Tannock 2006; Di Paolo and Bocci 2007). In addition, in these tumor regions, the concentrations of the key nutrients are also low, leading to marked gradients with higher cellular turnover rates close to blood vessels and lower cell proliferation rates (and cell cycle arrest) farther from the nearest microvessel before treatment and to repopulation of surviving tumor cells after/between treatments (Tannock 1968, 2001; Hirst and Denekamp 1979).

Cells dividing at a reduced rate would be protected from the effects of cytotoxic therapies whose activity is "selective" for rapidly dividing cell populations with a

Table 15.1. Role of chaotic tumor microcirculation in acquired treatment resistance (selection)

Pathophysiological condition	Leads via	To
Inadequate and heterogeneous perfusion	Inefficient and heterogeneous delivery of cytotoxic agents	Impaired pharmacokinetics of drugs, impaired delivery of therapeutic macromolecules and gene therapies
	Inefficient and heterogeneous nutrient supply yielding lower cell proliferation rates /cell cycle arrest	Protection from cytotoxic therapies whose activity is selective for rapidly dividing cells
Arterio-venous shunt vessels	Shunt perfusion (i.e., flow bypassing exchange vessels)	Impaired delivery of cytotoxic agents
Enlarged diffusion distances	Compromised penetration of cytotoxic agents	Insufficient concentrations of drugs and therapeutic macromolecules in tumor regions distant to patent blood vessels

short cell cycle, a large proportion of cells in S-phase and, therefore, a large growth fraction (Hall and Giaccia 2006; Trédan et al. 2007). There is a strong indication that the growth fraction decreases as tumor size increases, at least in experimental tumor systems.

Anti-angiogenic therapy for solid tumors using inhibition of VEGF-signaling can generate an early-phase of "normalization" of tumor vasculature (Jain 2001). This occurs via the recruitment of pericytes to the tumor microvasculature, an effect associated with a temporary, short-lived stabilization of the vessels and a (still hypothetic) improvement in blood flow. The latter may be accompanied by improved oxygen and drug delivery, creating a window of opportunity for higher sensitivity to ionizing radiation and the delivery of anti-cancer agents (Jain 2005). The postulated increase in pericyte recruitment is thought to be mediated by angiopoietin-1 and matrix metalloproteinases (Lin and Sessa 2004).

15.3 Interstitial Barriers to Delivery of Therapeutic Agents

As already outlined in Chap. 4, the interstitial compartment of tumors is significantly different from that of normal tissues. As a result of (a) vessel leakiness, (b) lack of functional lymphatics, (c) interstitial fibrosis and (d) contraction of the interstitial matrix mediated by stromal fibroblasts, most solid tumors have an increased interstitial (hydrostatic) fluid pressure (IFP; Jain 1987, 1990; Heldin et al. 2004; Milosevic et al. 2004; Cairns et al. 2006).

Increased interstitial fluid pressure (IFP) within solid tumors decreases extravasation and inhibits the extravascular transport of larger molecules (e.g., monoclonal antibodies, cytokines) by convection (see Table 15.2). Macromolecules rely more heavily on convection as opposed to simple diffusional transport. Interstitial transport of macromolecules is further impaired by a much denser network of collagen fibers in the extracellular matrix of tumors as compared to normal tissues. Collagen content in tumors is much higher and collagen fibers are much thicker than in normal tissues, leading to an increased mechanical stiffness of the tissue (Netti et al. 2000; Heldin et al. 2004).

IFP is almost uniform throughout a tumor and drops precipitously at the tumor/normal tissue interface. For this reason, the interstitial fluid oozes out of the tumor into the surrounding normal tissue and carries away anticancer agents with it (Fukumura and Jain 2007). As another consequence of this drop in IFP, blood may be diverted away from the tumor center toward the periphery where anticancer agents may be lost from larger vessels.

Transmural coupling between IFP and microvascular pressure can critically reduce perfusion pressure between up- and downstream tumor blood vessels (see Chap. 4, Sect. 4.6) leading to blood flow stasis and thus inadequate delivery of anticancer agents, in addition to the mechanisms impairing blood flow already mentioned.

Table 15.2. Interstitial barriers in acquired treatment resistance

Pathophysiological condition	Leads via	To
Interstitial hypertension	Decreased extravasation and compromised interstitial transport of macromolecules	Impaired delivery of therapeutic macromolecules (e.g., passive immunotherapy) and gene therapies, disturbed immigration of immune effector cells
Dense network of collagen fibers	Compromised interstitial transport of macromolecules	Impaired delivery of therapeutic macromolecules (e.g., passive immunotherapy)
IFP drop at the tumor/normal tissue interface	Centrifugal interstitial fluid flow Diversion of blood from tumor center to periphery	Loss of anticancer agents Loss of anticancer agents in the tumor periphery
Transmural coupling between IFP and microvascular pressure	Critical reduction in perfusion pressure	Flow stasis compromising intra-tumor pharmacokinetics
Expansion of the interstitial space	Increase in distribution space for anticancer (and diagnostic) agents	Time necessary for drug concentration equilibrium between vascular and interstitial space may be prolonged

IFP = interstitial fluid pressure

Interactions between cancer cells and the extracellular matrix can affect their response to chemotherapy. The basic mechanisms involved in the so-called adhesion-mediated drug resistance are rather complex and still under investigation. Agents that can modulate cell adhesion might enhance the effects of chemotherapy (Trédan et al. 2007).

Several types of treatment have been shown to decrease tumor IFP in patients (Lee et al. 2000; Willett et al. 2004, 2005; Batchelor et al. 2007). This decrease in IFP has been attributed to a substantial reduction in vascular permeability (concomitant with a pruning of tumor vessels) after angiogenesis-inhibiting treatment with VEGF-receptor inhibitors (combined with radiation and/or chemotherapy).

15.4 Hypoxia as an Obstacle in Tumor Therapy

Although resistance of human tumors to anticancer agents is mostly ascribed to gene mutations, gene amplification or epigenetic changes that influence the uptake, metabolism or export of drugs from single cells (Trédan et al. 2007), tumor hypoxia plays a pivotal role in acquired treatment resistance, since O_2 depletion in solid tumors is classically associated with resistance to radiotherapy, but has also been shown to diminish the efficacy of certain forms of chemotherapy, of photodynamic therapy, immunotherapy and hormonal therapy (for reviews since 2000, see Chaplin et al. 2000; Vaupel et al. 2001a,b, 2002, 2004; Vaupel and Mayer 2005; Shannon et al. 2003; Vaupel 2004b; Weinmann et al. 2004; Brown 2002, 2007; Tannock et al. 2005; Kurebayashi 2005; Hall and Giaccia 2006; Liao et al. 2007; Vaupel and Höckel 2008; Bristow and Hill 2008).

15.4.1 General Aspects of Hypoxia-Driven Treatment Resistance

Hypoxia protects tumor cells from damage by nonsurgical anticancer therapies that are directly or indirectly O_2-dependent (or both; for reviews see Moulder and Rockwell 1987; Durand 1991, 1994; Tannock and Hill 1992; Teicher 1993, 1994, 1995; Hall 1994; Vaupel 1997b; Chaplin et al. 2000; Höckel and Vaupel 2001; see Table 15.3).

Table 15.3. Tumor hypoxia and acquired treatment resistance (selection of mechanisms)

Treatment affected	Mechanisms involved	Examples	References
A. Direct effects			
X- and γ-rays*	Reduced "fixation" of DNA damage		Hall and Giaccia (2006)
Chemotherapy*	Reduced generation of free radicals	Antibiotics (bleomycin, doxorubin)	Erlichman (1992)
Photodynamic therapy	Reduced generation of free radicals		Shannon et al. (2003) Henderson and Fingar (1987)
B. Indirect effects			
X- and γ-rays*	Cell cycle effects, modulation of proliferation kinetics Increased activity of repair enzymes Enhanced expression of anti-apoptotic proteins Selection of apoptosis-resistant cells Elevated intracellular levels of glutathione and associated nucleophilic thiols		Hall and Giaccia (2006)
Chemotherapy**	Cell cycle effects, modulation of proliferation kinetics	Vinca alkaloids, methotrexate, platinum compounds, taxanes, doxorubicin	Chabner et al. (1996)
	Increased activity of repair enzymes	Alkylating agents, platinum compounds, etoposide, anthracyclines	Chabner et al. (1996) Zeller (1995)
	Elevated intracellular levels of glutathione	Melphalan	
	Increased telomerase activity	Telomerase inhibitors	Nishi et al. (2004) Anderson et al. (2006)
	Development of an aggressive phenotype		Lunt et al. (2008)
	Amplification and increased synthesis of dihydrofolate reductase (DHFR)	Methotrexate	Rice et al. (1986)
	Increased synthesis of growth factors (e.g., TGF-β, bFGF)		Wei and Au (2005)
	Increased transcription of membrane transporters (e.g., GP-170, GLUT-1)	Vinca alkaloids, anthracyclines, etoposide, taxanes	Vera et al. (1991) Comerford et al. (2002)
	Increased expression of anti-apoptotic proteins, selection of apoptosis-resistant cells	Alkylating agents, cisplatin, anthracyclines, etoposide	Cole and Tannock (2005)
	Protection against drug-induced senescence	Anthracyclines	Sullivan et al. (2008)

*Anemia acts as a factor worsening tumor hypoxia

**Anemia acts as a factor that intensifies tumor hypoxia and that may impair transport of some cytotoxic drugs by red blood cells

Table 15.3. (*continued*) Tumor hypoxia and acquired treatment resistance (selection of mechanisms)

Treatment affected	Mechanisms involved	Examples	References
B. Indirect effects			
Endocrine therapy	Reduced expression of estrogen receptor	Hormonal therapy of breast cancer	Kurebayashi (2005)
	Enhanced androgen receptor function	Androgen-deprivation therapy	Park et al. (2006)
Immunotherapy	Reduced survival and proliferation of T-cells		Kim et al. (2008)
	Reduced production of cytokines by T-cells		Lukashev et al. (2007)
	Immunosuppression by adenosine		Sitkovsky and Lukashev (2005)
	Tumor-associated macrophages recruited to hypoxic sites can switch to a "protumor phenotype" leading to immune evasion of tumors		Lewis and Murdoch (2005)

15.4.1.1 Direct Effects

Direct effects (i.e., effects of hypoxia per se) are mediated via deprivation of molecular O_2 and thus reduced generation of free radicals that some chemotherapeutic agents (e.g., the antibiotics bleomycin and doxorubicin; Erlichman 1992) and photodynamic therapy require to be maximally cytotoxic. Sparsely ionizing radiation (X- and γ-rays) needs O_2 for "fixation" of DNA damage (Fig. 15.1).

15.4.1.2 Indirect Effects Based on Changes in the Transcriptome, in Differential Regulation of Gene Expression and in Alteration of the Proteome

Indirect effects, which to a great extent are reversible and which may occur upon exposure to oxygen levels <1% (pO_2 <7 mmHg), rely on the hypoxia-mediated modulation (stimulation or inhibition) of gene expression (see Fig. 15.1) and posttranscriptional or posttranslational effects resulting in changes in the proteome and leading, inter alia, to

(a) modulation of proliferation kinetics, perturbations of the cell cycle distribution, the number of tumor cells accumulating in G_1-phase (e.g., 5-FU; Yoshiba et al. 2008) and a reduction in the fraction of active S-phase cells (e.g., the vinca alkaloids and methotrexate exhibit cell-cycle-phase specificity; Chabner et al. 1996). As a rule, the portion of proliferating cells decreases with increasing hypoxia and increasing duration of hypoxia. Hereby, the fraction of hypoxic and not proliferating—but still viable—tumor cells is of special interest;
(b) quantitative changes in cellular metabolism (e.g., intensified glycolysis in hypoxic tumors with tissue acidosis, which in turn can have an impact on cellular activation, intracellular accumulation and membrane transport of drugs), increased enzyme activities, elevated intracellular concentrations of glutathione (GSH) and associated nucleophilic thiols that can compete with the target DNA for alkylation (see Table 15.3);

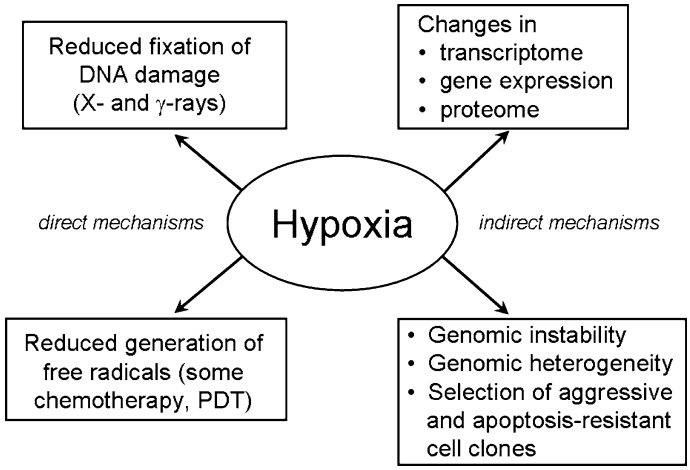

Fig. 15.1. Hypoxia-driven direct and indirect mechanisms leading to acquired treatment resistance

(c) increased transcription of membrane transporters (e.g., GLUT-1 facilitating the efflux of vinblastine, Vera et al. 1991), DNA repair enzymes, autocrine and paracrine growth factors (e.g., TGF-β), proteins involved in cell detachment and tumor invasiveness, and resistance-related proteins. Many hypoxia-inducible genes are controlled by the transcription factors HIF-1, nuclear factor κB (NFκB) and activator protein-1 (AP-1; Koong et al. 1994; Dachs and Tozer 2000; Laderoute et al. 2002).

In addition to hypoxia, other epigenetic microenvironmental factors (e.g., acidosis, glucose depletion, lactate accumulation) may also be involved in the mechanisms described above. (For more details on hypoxia-mediated proteome changes, see Rice et al. 1986; Laderoute et al. 1992; Ausserer et al. 1994; Graeber et al. 1994; Sanna and Rofstad 1994; Giaccia 1996; Mattern et al. 1996; Raleigh 1996; Brown and Giaccia 1998; Sutherland 1998; Semenza 2000a,b; Höckel and Vaupel 2001).

15.4.1.3 Indirect Effects Based on Enhanced Mutagenesis, Genomic Instability and Clonal Selection

Therapeutic resistance can also result from (progressive) genome changes and clonal selection at tissue O_2 concentrations <0.1% (pO_2 <0.7 mmHg; Vaupel 2004b, 2008).

Increasing resistance towards nonsurgical therapy concomitant with primary tumor growth can also be driven by transient or persistent genomic changes and clonal selection (often associated with subsequent clonal dominance) due to a hypoxia-related strong selection pressure (see Fig. 15.1). Hypoxia promotes genomic instability (through point mutations, gene amplification and chromosomal rearrangements), thus increasing the number of genetic variants and thereby promoting clonal and intrinsic tumor cell heterogeneity. Emancipative proliferation of resistant clonal variants in a "survival of the fittest" scenario and malignant progression are the final results (see Table 15.3).

Hypoxia-mediated clonal selection of tumor cells with persistent genomic changes can lead, inter alia, to a loss of differentiation and of apoptosis, which can stabilize or further aggravate tumor hypoxia and which in turn again promotes malignant progression (Vaupel 2004a, 2008). Thus, hypoxia is involved in a vicious circle that is regarded as a fundamental biologic mechanism of malignant disease (for reviews, see Höckel and Vaupel 2001; Vaupel et al. 2004; Vaupel 2008). Other consequences of hypoxia-induced malignant progression are an increased locoregional spread and enhanced metastasis (Höckel et al. 1996a, 1998). (For more details on hypoxia-mediated genome changes and expansion of aggressive tumor subclones, see Young et al. 1988; Stoler et al. 1992; Cheng and Loeb 1993; Stackpole et al. 1994; Russo et al. 1995; Giaccia 1996; Graeber et al. 1996; Reynolds et al. 1996; Kim et al. 1997; Höckel et al. 1999; Höckel and Vaupel 2001).

15.4.2 Tumor Hypoxia as an Obstacle in Radiotherapy

Tumor hypoxia may present a severe problem for radiation therapy (X- and γ-radiation), because radiosensitivity is progressively limited when the O_2 partial pressure in a tumor is less than 25–30 mmHg, the latter representing the median O_2 tensions in most normal tissues (Vaupel et al. 2003; see Fig. 15.2). Hypoxia-associated resistance to photon radiotherapy is multifactorial. Molecular oxygen "fixes" (i.e., makes permanent) DNA damage produced by oxygen free radicals, which arise after the interaction of radiation with intracellular water (Hall and Giaccia 2006). Thus, because of this so-called "oxygen-enhancement effect," the radiation dose required to achieve the same biologic effect is approximately three times higher in the absence of oxygen than in the presence of normal levels of oxygen (Gray et al. 1953). Evidence suggests that hypoxia-induced proteome and genome changes (see Table 15.3) may also have a substantial impact on radioresistance by increasing the levels of heat shock proteins and repair enzymes or by increasing the number of cells in a tumor with diminished apoptotic potential or increased proliferation potential of selected clones, both of which have been linked to radioresistance (for a review, see Höckel et al. 1996b; Höckel and Vaupel 2001).

Numerous clinical studies report an impaired radiocurability of anemic patients, most probably due to hypoxia-related radioresistance (Evans and Bergsjø1965; Bush 1986; Frommhold et al. 1998; Henke et al. 1999; Grau and Overgaard 2000; Kumar 2000; Harrison et al. 2002; Dunst 2004; Dunst and Molls 2008; Harrison and Blackwell 2004; Nowrousian et al. 2008; Haugen et al. 2004; Hu and Harrison 2005; Ludwig 2004; Prosnitz et al. 2005). A significant influence of hemoglobin level on the outcome of radiotherapy has been convincingly documented for carcinomas of the uterine cervix, head and neck, bladder and bronchus (for a review, see Grau and Overgaard 2000). One major reason for these observations may be the fact that anemia can strongly aggravate tumor hypoxia (Vaupel et al. 2006).

Carbon monoxide (CO) in tobacco smoke strongly binds to hemoglobin (formation of carboxyhemoglobin HbCO) and thus decreases the amount of "effective" hemoglobin. Furthermore, CO increases the hemoglobin affinity for O_2. The sum of these effects is a significant increase in tumor hypoxia and in radioresistance, resulting in a poorer treatment outcome after primary radiotherapy (for a review, see Grau and Overgaard 2000).

15.4.3 Tumor Hypoxia as an Adverse Parameter in Chemotherapy

Besides restricted delivery and uneven distribution (due to poor and heterogeneous blood flow) as well as reduced diffusional flux (due to enlarged diffusion distances), oxygen-dependency has been documented for a broad range of cytotoxic drugs (e.g., cyclophosphamide, carboplatin and doxorubicin) under in vitro and in vivo conditions (Teicher et al. 1981, 1990; Teicher 1994, 1995). However, these investigations have been qualitative, and clear hypoxic thresholds for O_2-dependent anticancer agents are still not available, although they presumably exist for each agent (Wouters et al. 2007). Thus, additional research is necessary to provide quantitative data on hypoxia-induced chemoresistance, although this information may be difficult to

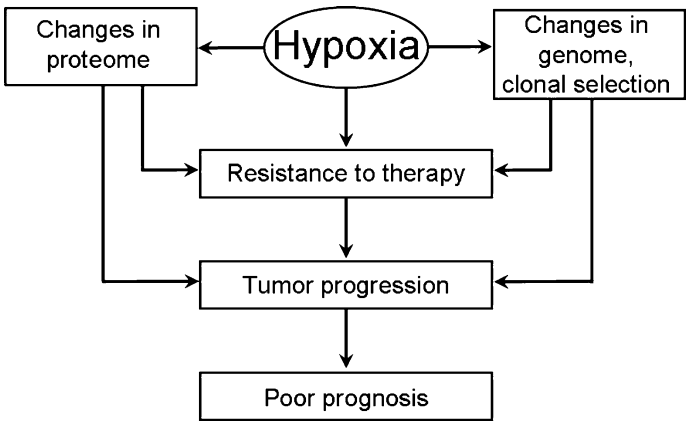

Fig. 15.2. Schematic representation of major hypoxia-induced mechanisms causing treatment resistance and malignant progression, finally leading to poor long-term prognosis

obtain under in vivo conditions. Multiple (direct and indirect) mechanisms are probably also involved in the hypoxia-induced resistance to chemotherapeutic agents, including a reduced generation of free radicals (e.g., bleomycin, anthracyclines), the increased production of nucleophilic substances such as glutathione, which can compete with the target DNA for alkylation (e.g., in the acquired resistance to alkylating agents), an increased activity of DNA repair enzymes (e.g., alkylating agents, platinum compounds; Chabner et al. 1996), an inhibition of cell proliferation and tissue acidosis, which is often observed in hypoxic tumors with a high glycolytic rate (Durand 1991, 1994). Furthermore, hypoxic stress proteins, the loss of apoptotic potential and multi-drug resistance proteins can impart resistance to certain chemotherapeutic drugs (Sakata et al. 1991; Hickman et al. 1994; Shannon et al. 2003). Clear hypoxic thresholds for chemotherapeutic agents are still not available, although resistance of hypoxic cells to conventional chemotherapy is well documented (Wouters et al. 2007).

Anemia is an independent risk factor for survival in most cancers treated with chemotherapy (e.g., Harrison and Blackwell 2004; Ludwig 2004; Prosnitz et al. 2005; Van Belle and Cocquyt 2003). As with radiotherapy, the presence of anemia and its association with inferior results of chemotherapy may be—at least partially—linked to severe hypoxia and its profound effect on tumor biology (e.g., development of an aggressive phenotype). However, anemia as a result of a reduced red blood cell mass may also have a negative impact on the pharmacokinetics of chemotherapeutic agents (Nowrousian 2008). RBCs have been reported to play an important role in storage, transport and metabolism of particular cytotoxic drugs. Anthracyclines, ifosfamide and its metabolites, and topoisomerase I/II inhibitors are incorporated in erythrocytes and may be transported by these cells to the tumor tissue and mobilized by active or passive mechanisms (Highley et al. 1997; Ramanathan-Girish and Boroujerdi 2001; Schrijvers 2003). 6-Mercaptopurine, methotrexate and aminotrexate are reported to accumulate in erythrocytes (Cole et al. 2006; Halonen et al. 2006). As shown for oxaliplatin, platinum-derived cytotoxic agents are also bound to erythrocytes and transported by RBCs (Luo et al. 1999). In an animal model, a significant correlation was found between concentrations of melphalan in erythrocytes and the tumor availability of this drug (Wildiers et al. 2002). Because of their potential ability to take up, transport and deliver various antineoplastic drugs, erythrocytes have increasingly become interesting objects to be evaluated as biological carriers in clinical oncology. Pretreatment elevation and/or maintenance of Hb levels are therefore essential, irrespective of the way in which this goal is achieved (Wildiers et al. 2002).

15.4.4 Tumor Hypoxia as an Obstacle in Chemoradiation

The combination of radiotherapy and chemotherapy is a promising approach because of its independent cell kill effect and the property of some cytotoxic agents to enhance the effect of radiotherapy. At the end of the 1970s, platinum complexes were described as being able to act as potent radiosensitizers of hypoxic tumor cells (Douple and Richmond 1978, 1979). As an obstacle in this type of chemoradiation, Koukourakis et al. (2002) have suggested that (hypoxia-induced?) overexpression of HIF-1α in patients with head and neck cancer may be related to substantial resistance to carboplatin chemoradiotherapy. More in-depth research is needed to accurately characterize adverse effects of hypoxia in this type of combination therapy.

15.4.5 Tumor Hypoxia as a Barrier for Other Nonsurgical Anticancer Therapies

15.4.5.1 Photodynamic Therapy

Photodynamic therapy-mediated cell death requires the presence of oxygen, a photosensitizing drug, and light of the appropriate wavelength, both in vitro and in vivo (for a review see Freitas and Baronzio 1991). However, reports vary greatly on the extent to which photodynamic therapy with hematoporphyrin derivatives is dependent on oxygen (Moan and Sommer 1985; Henderson and Fingar 1987). Cells were not killed under anoxic conditions. The critical threshold—below which progressively reduced cell death was observed—varied between 15 and 35 mmHg (Mitchell et al. 1985; Henderson and Fingar 1987; Chapman et al. 1991), probably because of reduced production of singlet oxygen species ($^1O_2$) and different sensitivities from the treatment in different cell lines. Considering the reduced effectiveness of photodynamic agents at lower O_2 partial pressures, the rapid induction of tumor hypoxia by photodynamic therapy itself—either as a consequence of a photodynamic therapy-induced decrease in blood flow or as a result of oxygen consumption by the photodynamic therapy process itself—has

to be considered under in vivo conditions, since it may mean that this therapy is self-limiting (Chapman et al. 1991; Chen et al. 2002). Photodynamic therapy involving prodrugs, such as aminolevulinic acid (ALA), may be further limited because conversion of the prodrug to the active photosensitizer appears to be less effective under hypoxic conditions.

15.4.5.2 Immunotherapy

As already described in Sects. 15.2 and 15.3, immunotherapy is heavily hampered by the morphologically aberrant tumor microvasculature and increased interstitial fluid pressure, which can impede the delivery of cytokines and monoclonal antibodies and can prevent immigration of immune effector cells into the established tumor parenchyma.

Tumor hypoxia can dramatically impede the effectiveness of certain (passive) immunotherapies using cytokines (interferon-γ and tumor necrosis factor-α). Hypoxia also reduces survival and proliferation of T-lymphocytes and the production of cytokines by these cells (Kim et al. 2008; Lukashev et al. 2007). Pharmacological studies have firmly established that high levels of adenosine, a pathophysiological feature of solid tumors (see Chap. 4, Sect. 4.11.12), have immunosuppressive effects (Sitkovsky and Lukashev 2005; Ohta et al. 2006). In addition, hypoxia can alter IL-2-induced activation of lymphokine-activated killer (LAK) cells (reviewed by Chaplin et al. 2000; Kim et al. 2008; Sitkovsky and Lukashev 2005). The potency of treatment started to decrease at oxygen partial pressures of less than approximately 35 mmHg (≈ 5% O_2).

15.4.5.3 Resistance to Hormonal Treatment

Endocrine therapy is the treatment of choice for patients with breast cancer expressing estrogen receptor (ER) and/or progesterone receptor (PR). A hypoxic microenvironment has been shown to posttranscriptionally reduce ER-α expression in breast cancer cells and thus decreases sensitivity to hormonal agents. ER-α-negative invasive breast cancer is more aggressive and in situ cancer is associated with increased risk of progression to invasive disease (Kurebayashi et al. 2001; Kurebayashi 2005; Helczynska et al. 2003; Stoner et al. 2002). Cooper et al. (2004) have shown that the reduced ER-α expression in breast cancers is caused by persistent changes in proteasome function as a response to intermittent hypoxia. As a consequence, the latter authors observed a diminished response to estradiol and development of resistance to endocrine therapy.

15.5 Tumor Acidosis and Treatment Resistance

As already outlined in Chap. 4, tumor cells have a lower extracellular pH (pH_e) than normal cells. This is an inherent characteristic of the tumor phenotype. Like normal cells, tumor cells have a neutral to slightly alkaline cytosolic ("internal") pH (pH_i), which is considered to be permissive for cell proliferation (Gillies et al. 1992). The result is a reverse (or negative) pH gradient ($pH_i > pH_e$) across the tumor-cell plasma membrane in vivo compared with normal tissues where $pH_i < pH_e$ (≈ 7.2 vs. ≈ 7.4; reviewed in Vaupel et al 1989; Griffiths 1991).

The extracellular acidosis in tumors is not simply caused by excessive production of lactic acid and CO_2, but may also be the result of other mechanisms yielding H^+ ions that are exported into the extracellular space mainly via the H^+-monocarboxylate cotransporter (MCT1) and the Na^+/H^+ antiporter (NHE1), and—to a lesser extent—by a vacuolar type H^+-pump (H^+-ATPase; Fais et al. 2007). Taking the various H^+ sources of the tumor metabolism into account, it is not surprising that hypoxia is not always correlated with a decrease in extracellular pH, i.e., acidic tumor regions and hypoxic tumor areas are not necessarily congruent.

pH effects on therapeutic modalities were summarized extensively prior to 2000 by Wike-Hooley et al. (1984), Tannock and Rotin (1989), Durand (1991,1994), Song et al. (1993, 1999), Vaupel (1997), Gerweck (1998) and Stubbs (1998). More recent reviews include Stubbs et al. (2000), Evelhoch (2001) and Roepe (2001).

15.5.1 Effects of Tumor Acidosis on Ionizing Radiation

Cell survival after ionizing radiation has been assessed at low extracellular pH for several mammalian cell lines. The results demonstrated increased radiation resistance at reduced pH_e, the effect, however, being much less than that due to hypoxia (Haveman 1980; Röttinger

et al. 1980; Freeman et al. 1981; Röttinger and Mendonca 1982). The mechanisms involved may be due to either a greater capacity for DNA repair under low pH conditions or to an inhibition of the fixation of potentially lethal radiation damage (Freeman and Sierra 1984; Tannock and Rotin 1989). Furthermore, it has been reported that an acidic environment can suppress radiation-induced postmitotic apoptosis (Lee et al. 1987).

Low environmental pH has also been shown to inhibit cell proliferation, can exert substantial effects on cell cycle that also modify radiosensitivity and can select for a more aggressive phenotype (Hill et al. 2001; Rofstad et al. 2006; see Table 15.4).

Table 15.4. Tumor acidosis and acquired treatment resistance (selection of mechanisms)

Treatment affected	Mechanisms involved	Examples	References
X- and γ-rays	Reduced "fixation" of DNA damage		Freeman and Sierra (1984)
	Increased capacity for DNA repair		Haveman (1980) Röttinger et al. (1980)
	Cell cycle effects, reduced cell proliferation rate		Taylor and Hodson (1984) Eagle (1973)
	Development of an aggressive phenotype		Hill et al. (2001) Rofstad et al. (2006)
	Suppression of radiation-induced apoptosis		Lee et al. (1987)
Chemotherapy	Cell cycle effects, reduced cell proliferation rate		Wike-Hooley et al. (1984) Cole and Tannock (2005) Valeriote and van Putten (1975)
	Reduced active uptake due to ATP-depletion Reduced uptake by diffusion	Methotrexate Weakly basic drugs	Gerweck and Seetharaman (1996)
	Increased DNA repair	Alkylating agents	Sarkaria et al. (2008)
	Over-expression of P-glycoprotein (P_{gp}), increased drug efflux	Anthracyclines vinca alkaloids	Wei and Roepe (1994) Lotz et al. (2007)
	Resistance to apoptosis	Overexpression of P_{gp}	Robinson et al. (1997)
Immunotherapy	Inhibition of cell-mediated anti-tumor immunity		Lardner (2001)
	Decreased T-cell-mediated cytotoxicity through P_{gp}.overexpression		Weisburg et al. (1996)
	Inhibition of LAK-cells		Severin et al. (1994)
	Depression of NK-cells		Loeffler et al. (1991)

15.5.2 pH and Chemotherapy

The transport of drugs into tumor cells (either by diffusion or carrier-mediated mechanisms) and their intracellular metabolism are pH-dependent (Tannock and Rotin 1989). Since the cellular uptake of drugs by diffusion is efficient only for the non-ionized form of compounds and since the extracellular pH in tumors is acidic with the cytosolic pH being maintained in the neutral/slightly alkaline range, the respective pH gradient acts to exclude weakly basic drugs and thus impairs their cellular uptake by diffusion. Since cell membranes are readily permeable only to uncharged drug molecules, weak bases tend to concentrate on the more acid side of the membrane, i.e., in the extracellular space, while weak acids accumulate on the more alkaline side of the membrane, i.e., in the cytosolic compartment. Weakly basic drugs include doxorubicin, idarubicin, epirubicin, daunarubicin, bleomycin, mitoxantrone and vinca alkaloids (Raghunand and Gillies 2000, 2001; Gerweck and Seetharaman 1996; Gerweck 1998; Gerweck et al. 2006).

Multiple indirect mechanisms may additionally be involved in the acidosis-induced resistance to chemotherapeutic agents, including an increased efflux of drugs (Wei and Roepe 1994) and resistance to apoptosis (Robinson et al. 1997), the latter mechanisms being mediated by overexpression of P-glycoprotein. Furthermore, an increased activity of DNA repair enzymes has been convincingly described (Sarkaria et al. 2008), and an inhibition of cell proliferation and cell cycle effects have extensively been discussed as mechanisms reducing the effectiveness of chemotherapeutic agents in acidic environments (e.g., Valeriote and van Putten 1975; see Table 15.4).

15.5.3 pH and Immunotherapy

Although there are only relatively few studies on the effect of acidic extracellular pH on immune cells and their function, evidence of impaired lymphocyte cytotoxicity and proliferation at acidic pH is beginning to emerge (for a review see Lardner 2001). There is a growing awareness among immunologists and oncologists of the potential modulatory role of the acidic tumor microenvironment on immune cell function (see Table 15.4). The majority of the work to date has focused primarily on cell-mediated immunity, with only a few studies on humoral immunity. Summarizing the few data available so far, the acidic microenvironment may be inhibitory to the antitumor immunity (Cairns et al. 2006). Most of this evidence is experimental, and clinical demonstration of similar phenomena will be difficult. Furthermore, many data are still too preliminary for firm conclusions to be made and are thus speculative.

15.6 Conclusions

Besides "classical" drug resistance (mostly based on the molecular biology of tumors), which can only partly explain the lack of treatment efficacy, acquired therapeutic resistance due to the impact of hostile microenvironmental conditions is increasingly receiving attention in clinical practice. One of the goals of translational cancer research is to obtain a better understanding of the impact of these hostile microenvironmental parameters on tumor response to therapy, in order to improve patients' outcomes.

Based on the association between hostile microenvironmental parameters and treatment failure, further development and validation of noninvasive techniques for the repeated assessment of these factors are urgently needed to enable an application in the clinical routine and integration into general patient care. Pretreatment assessment of the hostile micromilieu and the pathophysiology of individual tumors should allow a selection of patients for more aggressive treatment and/or for individualization of therapy.

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