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Receptor-mediated and enzyme-dependent targeting of cytotoxic anticancer drugs

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Abstract

This review is a survey of various approaches to targeting cytotoxic anticancer drugs to tumors primarily through biomolecules expressed by cancer cells or associated vasculature and stroma. These include monoclonal antibody immunoconjugates; enzyme prodrug therapies, such as antibody-directed enzyme prodrug therapy, gene-directed enzyme prodrug therapy, and bacterial-directed enzyme prodrug therapy; and metabolism-based therapies that seek to exploit increased tumor expression of, e.g., proteases, low-density lipoprotein receptors, hormones, and adhesion molecules. Following a discussion of factors that positively and negatively affect drug delivery to solid tumors, we concentrate on a mechanistic understanding of selective drug release or generation at the tumor site.

Keywords: Antitumor; Antibody; Prodrug; Drug delivery; Immunoconjugate; Drug release

Abbreviations: Ab, antibody; ADAPT, antibody-directed abzyme prodrug therapy; ADC, antibody-directed catalysis; ADEPT, antibody-directed enzyme prodrug therapy; Ag, antigen; AP, alkaline phosphatase; AraC, cytarabine; BDEPT, bacterial-directed enzyme prodrug therapy; BSA, bovine serum albumin; CB1954, 5-(1-aziridinyl)-2,4-dinitrobenzamide; CCM, 7-(4-carboxybutanamido)cephalosporin-phenylenediamine; CD, cytosine deaminase; CEA, carcinoembryonic antigen; CHO, aldehyde group; Cit, L-citrulline; CMDA, 4-[*N*-(2-chloroethyl)-*N*-[2-(mesyloxy)ethyl]amino]benzoyl-L-glutamic acid; CP, carboxypeptidase; DAVLB, desacetylvinblastine; DAVLBHYD, 4-desacetylvinblastine-3-carbohydrazide; dCK, deoxycytidine kinase; Dex, dextran; DM1, maytansine derivative; DMT, *p*,*p*′-dimethoxytrityl; DNM, daunomycin; DNR, daunorubicin; DOX, doxorubicin; DSG, 15-deoxyspergualin; DTT, dithiothreitol; EGF, epidermal growth factor; EP, etoposide phosphate; Fab, monovalent antibody fragment; $F(ab')_2$, bivalent antibody fragment; 5-FdU, 5-fluorodeoxyuridine; 5-FC, 5-fluorocytosine; FdUR, 5-fluoro-2′-deoxyuridine; 5-FU, 5-fluorouracil; GB, guanidinobenzoatase; GCV, ganciclovir; GDEPT, gene-directed enzyme prodrug therapy; GI, gastrointestinal; GUS, glucuronidase; HAMA, human anti-mouse antibody; HDL, high-density lipoprotein; HPMA, *N*-(2-hydroxypropyl)methacrylamide; HSA, human serum albumin; HSV, herpes simplex virus; IDA, idarubicin; IgG, immunoglobulin-γ; IL, interleukin; ING-1, anti-Epcam; LDL, low-density lipoprotein; Le^x or Le^y, Lewis x or y; mAb, monoclonal antibody; MDR, multidrug resistance; MeP, 6-methylpurine; MeP-dR, 6-methylpurinedeoxyriboside; MeT, *p*-monomethyltrityl; MMC, mitomycin C; MMCDan, anionically charged polymeric prodrug of mitomycin C; MMT, *p*-monomethoxytrityl; morph-DOX, morpholinodoxorubicin; MR, mole ratio; MTD, maximum tolerated dose; MTX, methotrexate; *N*-AcMEL, *N*-acetylmelphalan; NCS, neocarzinostatin; NTR, nitroreductase; PABC, *p*-aminobenzylcarbonyl; PDM, phenylenediamine mustard; PEG, polyethylene glycol; PGP, P-glycoprotein; PHEG, poly[N^5-(2-hydroxyethyl)-L-glutamine]; PNP, purine nucleoside phosphorylase; Pt, platinum; RGD, Arg-Gly-Asp; SPDP, *N*-succinimidyl 3-(2-pyridyldithio)-propionate; Tk, thymidine kinase; TX, 6-thioxanthine; VEGF, vascular endothelial growth factor; XGPRT, xanthine-guanine phosphoribosyltransferase.

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1. Introduction

Despite several decades of intensive research in the laboratory and the clinic, the long-term outlook for cancer patients with aggressive disease remains discouraging (Brun et al., 1997; Dunton, 1997; Piccart, 1996; Rahman et al., 1997). Unlike bacteria and viruses, cancer cells do not contain molecular targets that are completely foreign to the host. As a result, cytotoxic anticancer therapy has relied primarily on the enhanced proliferative rate of cancer cells, using drugs that act on DNA, tubulin, and enzymes such as the topoisomerases that are important in DNA replication. However, for patients with appreciable tumor burdens, clinically approved cytotoxics usually only cause remissions of limited duration and variable degree, followed by regrowth and spread of often more malignant and multidrug-resistant disease (Eltahir et al., 1998). Part of the reason for this is that hypoxic cells in the center of tumors can be essentially dormant and much less susceptible to traditional cancer drugs (Clarkson, 1974), not only because they are temporarily in a growth-arrested state, but also because of limited drug penetration (Erlanson et al., 1992) and induced cellular resistance mechanisms (Wartenberg et al., 1998). When these cells are revived by vascularization, following destruction of the tumor periphery, there is evidence that they often have a higher metastatic potential (Young & Hill, 1990; Young et al., 1988). In addition, aggressive microme-

tastases and minimal residual disease (Hirsch-Ginsberg, 1998), often beginning only as vanishingly small populations of cells that evade resection of the primary tumor or first-line chemotherapy, are often the cause of clinical relapse (Schott et al., 1998). These stray cells, which are difficult to detect, can also be present as contamination in autologous grafts after high-dose chemotherapy (Ross, 1998). Newer approaches to cancer chemotherapy that exploit angiogenesis, tumor suppressors, and other signal transduction pathways show promise, but have yet to make an impact in the clinic (Alessandro et al., 1996; OReilly, 1997; Schwartz, 1996; Sebti & Hamilton, 1997).

It can be argued that many of the shortcomings of currently approved cytotoxics are a result of dose-limiting toxic side effects, not only toward normally proliferative cell populations (Lowenthal & Eaton, 1996), but also, in the case of specific classes of chemotherapeutics, organ-specific toxicities such as the cardiotoxicity shown by most members of the widely used anthracycline family of anticancer agents (Hortobagyi, 1997; Shan et al., 1996). This effectively limits the amount of agent that can be given to below the threshold that exposes all the tumor tissue to a killing dose, resulting in induction of resistance mechanisms and metastasis. In the past several decades, various approaches toward targeting cytotoxic agents to cancer cells have been developed that use conjugated forms of these agents with carriers that selectively accumulate in tumors. The best of these approaches combine a protective mechanism for normal tissues that deactivates the agent until the tumor is reached, at which time, a tumor-specific mechanism releases the cytotoxic effect. Therefore, the goal of targeting is 2-fold: to actively deliver an effective dose of a cytotoxic agent to tumor tissue and to protect the rest of the body from its toxic effects.

This review will survey various approaches to targeting cytotoxic drugs to neoplastic tissue, using vehicles that show affinity for specific biomolecules expressed on the surface of cancer cells or in tumor-associated tissue, such as vasculature and stroma. It will emphasize the rational design of drug release mechanisms that take advantage of conditions at the tumor site or within cancer cells. It will not cover the following areas, for which the reader is directed to recent reviews or leading articles: delivery of protein toxins (Ghetie & Vitetta, 1994; Pastan, 1997), radioimmunotherapy (Schott et al., 1994), boron-neutron capture therapy (Chen et al., 1997; Mehta & Lu, 1996), targeted photodynamic therapy (Akhlynina et al., 1997; Peterson et al., 1996), electrochemotherapy (Jaroszeski et al., 1997), drug delivery using magnetic particles (Devineni et al., 1995; Lubbe et al., 1996), T-lymphocyte targeting using bacterial superantigens (Giantonio et al., 1997; Hansson et al., 1997) and bi-specific antibodies (Abs) (Mokotoff et al., 1996; Renner & Pfreundschuh, 1995), and passive targeting using liposomes (Ceh et al., 1997; Sharma & Sharma, 1997) and polymers (Cummings, 1998; Soyez et al., 1996; Zalipsky, 1995).

2. Properties of tumors that affect drug-carrier therapy

2.1. Tumor-associated antigens

The delivery of immunoconjugates to tumor-associated antigens (Ags) has been the most commonly employed method of anticancer targeting in preclinical studies. Cancer cells overexpress many proteins in comparison with normal tissue, as a result of their transformed state. Modern hybridoma technology has allowed the large-scale production of monoclonal antibodies (mAbs) raised to numerous tumor-associated Ags (Hellstrom & Hellstrom, 1991, 1997; Urban & Schreiber, 1992; Wick & Groner, 1997; Wright, 1984). Most Ags used for targeting are expressed to a lesser, varying degree in some normal tissues. If these molecules are receptors for growth factors or are differentiation related, for example, they may also be expressed in normal proliferative tissues, such as portions of the lining of the gastrointestinal (GI) tract. This expression can show striking interspecies differences. As such, these Ags are tumor-selective rather than tumor-specific, and therapy will target those normal tissues to some extent. In several clinical trials, this cross-reactivity with normal tissue has determined the maximum-tolerated dose (MTD) (Elias et al., 1990; Sugerman et al., 1995).

One of the first selective tumor markers discovered was the carcinoembryonic antigen (CEA), which is most likely a cell adhesion molecule (Johnson, 1992). Found in tumors associated with the GI tract, as well as some lung and breast cancers, CEA has been frequently targeted (Ballesta et al., 1995; Siler et al., 1993). Other notable classes of tumor-associated Ags that have been used in immunotherapy include α-fetoprotein (Masuda et al., 1994), gangliosides (Zhang et al., 1997a) such as the L6 Ag (Fell et al., 1992; Hellstrom et al., 1986), blood group carbohydrates (Ragupathi, 1996; Zhang et al., 1997b) such as Lewis y (Le^y) (recognized by the mAbs BR64 and BR96 and possibly related to apoptosis [Nagai et al., 1995] or cell migration [Garrigues et al., 1994; Hellstrom et al., 1990]), B-cell differentiation Ag (Rowland et al., 1993), the transferrin receptor (Starling et al., 1988), the adenocarcinoma-related KS1/4 (Bumol et al., 1988b; Varki et al., 1984), mucins (Hinman et al., 1993), selectins (Ravindranath et al., 1997), glycosphingolipids (Hakomori & Zhang, 1997), integrins (Ruoslahti, 1997), and other adhesion molecules (Chang & Pastan, 1996; Huang, Y. W. et al., 1997; Lally et al., 1997).

Ags that are more tumor selective recently have been found in oncogenic protein products (Appleman & Frey, 1996; Curiel, 1997; Halpern, 1997), such as the HER-2/neu (or c-erbB2) glycoproteins (Cirisano & Karlan, 1996; Disis & Cheever, 1997), or products resulting from chromosomal translocations (Rabbitts, 1994). The mutated form of the tumor suppressor p53 has been shown to be a tumor-specific Ag in T-cell targeting (Theobald et al., 1995), and a number of heat shock proteins have been found to be overexpressed in certain cancers, and may be sites that attract natural killer cell activity (Multhoff et al., 1997).

The receptor for folic acid (Coney et al., 1994) and the iron transport protein transferrin are overexpressed in many cancers (Lally et al., 1997; Richardson & Ponka, 1997), and the presence of transferrin in the blood-brain barrier has also allowed brain penetration of transferrin conjugates (Youle, 1996). The multidrug resistance (MDR)-associated membrane pump protein P-glycoprotein (PGP) has also been shown to be antigenic in a bi-specific Ab approach to target T-lymphocytes (Van Dijk et al., 1989) and immunotoxins (Bruggemann et al., 1991) to drug-resistant tumor cells. Recently, Ags associated with various cancers, such as melanoma (Merimsky et al., 1994), ovarian carcinoma (Bast et al., 1994), and gliomas (Kurpad et al., 1995), as well as growth factor-associated Ags (Fan & Mendelsohn, 1998), have been reviewed. In addition, the use of overexpressed cellular receptors for drug targeting has been reviewed (Feener & King, 1998).

Two problems related to targeting tumor-associated Ags are heterogeneity of Ag expression and Ag shedding. The origin of the first problem is complex, but may result in part from the genetic instability of cells in the necrotic region of tumor tissue (Fleuren et al., 1995; Reynolds et al., 1996). A given Ag can also be expressed with different glycosylation patterns within tumor tissue, leading to diminished or nonexistent Ab reactivity in certain areas (Hernando et al., 1994). Interferons (Guadagni et al., 1994; Murray, 1992; Schlom et al., 1990) have been used to enhance expression of certain tumor Ags in vitro and in vivo, and Ab-directed interleukin (IL)-2 has been proposed as an approach to overcome Ag heterogeneity by recruiting a host immune response against the tumor (Becker et al., 1996). Ag heterogeneity may not be a problem in cases where the cytotoxic drug is stable enough, and is delivered in sufficient quantity to kill Ag-negative cells by the "bystander effect." This is operative when excess drug in dead, Ag-positive cells is released into the tumor interstitium to be absorbed nonselectively (Laguzza et al., 1989; Liu et al., 1996; Niculescu-Duvaz et al., 1998). Other workers have addressed the potential problem of a "binding-site barrier," where a combination of high Ag expression and a tightly binding mAb may lead to reduced tumor penetration of an immunoconjugate (Shockley et al., 1992; Sung et al., 1992; 1993; van Osdol et al., 1991).

Ag shedding is related to the fact that tumor cells shed various biomolecules without the degree of control exerted on normal cells (Kiessling & Gordron, 1998). Some of these, such as adhesion molecules and proteases, are part of the metastatic cascade in which cancer cells dissociate from the primary tumor and degrade surrounding basement membrane (Taylor & Black, 1985). Instances of clinical detection of circulating tumor-associated Ag have been reported (Maimonis et al., 1990; van Hof et al., 1996), and in the case of melanoma, the degree of shedding has been linked to circulating cytokine levels (Anichini et al., 1993). Shed Ags can be expected to compete with tumor cell-bound Ags (van Hof et al., 1996), especially since they may be more accessible to the immunoconjugate. This will decrease the effectiveness of targeting therapy by the formation of inactive conjugate-Ag complexes that are rapidly cleared (Pimm et al., 1989).

2.2. Internalization

The importance of internalization of Ag-bound immunoconjugates by receptor-mediated endocytosis (Kato et al., 1996; Mellman, 1996), resulting in conjugate processing in endosomes and lysosomes, depends on the type of therapy. For antibody-directed enzyme prodrug therapy (ADEPT), in which an Ab-bound enzyme is localized to the cell surface where it unmasks a prodrug, internalization is not desired. For delivery of cytotoxic radioisotopes or photosensitizers, internalization probably does not matter. However, in general, it has been found that delivery of cytotoxic drugs or protein toxins is much more effective when the metabolic potential of endosomes and lysosomes can be utilized for drug release. The limitations of endocytosis as an entry point for drugs into cells depend on such factors as cell surface Ag density, rate of internalization, and re-expression (Kato & Sugiyama, 1997).

2.3. Tumor blood vessels and drug penetration

Tumor blood vessels possess a number of properties that differentiate them from those in normal tissue. Vasculature in well-differentiated tumors can be close to normal. However, in rapidly growing and large solid tumors, new blood vessels are often deficient in many ways, including interrupted or absent basement membranes and endothelial lining (Cobb, 1989); tortuous, often spiral-shaped, paths (Baish et al., 1996; Jain, 1994); lack of regularity and systematic connectivity leaving unvascularized areas, especially in the tumor interior, all together resulting in unstable blood flow (Eskey et al., 1994; Vaupel et al., 1989). Intercellular adhesion between tumor vascular endothelial cells is often poor, resulting in a high proportion of leaky blood vessels. In addition, large solid tumors generally do not develop a functional lymphatic network and, therefore, do not have adequate fluid drainage. One result of this is a sieving effect in which large molecules can become trapped in tumor tissue. These properties have been exploited in the "passive targeting" (Maeda, 1992) of polymer-bound drugs (Maeda et al., 1992; Seymour et al., 1995, 1996; Steyger et al., 1996), liposomes (Forssen, 1997; Gabizon et al., 1997; Uchiyama et al., 1995; Unezaki et al., 1996), nanoparticles (Hodoshima et al., 1997; Kwon & Okano, 1996), and nonspecific protein conjugates (Hunerbein et al., 1991; Suda et al., 1993). In one study, liposomes up to 400 nm in diameter were shown to passively diffuse through gaps in tumor blood vessels in LS174T adenocarcinoma xenografts in nude mice (Yuan et al., 1995).

These vascular abnormalities, exacerbated by constant angiogenesis, can result in a buildup of microvascular pressure (Boucher et al., 1996). Combined with a highly prolif-

erative cell population in a restricted space, the result can be a sizable positive osmotic intratumoral pressure and a net outflow of liquid from solid tumors into the surrounding tissue (Jain, 1996). This physical stress can also cause the virtual collapse of blood vessels and any functional lymphatics within tumors, further impairing blood flow (Helmlinger et al., 1997a). The barrier to the diffusion of molecules from the blood vessel, through the interstitium, to tumor cells has been shown to increase with tumor size and to be highly dependent on molecular weight, allowing small molecule drugs to penetrate much deeper into the tumor than proteins, polymers, or other particles (Yuan et al., 1995). This pressure gradient in solid tumors, along with the presence of significant areas lacking adequate vascularization, is an important consideration in the choice of, for example, a full-size Ab or a smaller Ab fragment as a delivery vehicle or an Ab with high or moderate binding affinity to an Ag (Baxter & Jain, 1991). Agents such as nicotinamide (Lee et al., 1992), pentoxifylline (Lee et al., 1994), hydralazine (Zlotecki et al., 1995), and tumor necrosis factor α (Kristensen et al., 1996) have been shown to increase tumor perfusion by reducing interstitial fluid pressure. In addition, Ab-targeted IL-2 was shown to increase tumor vascular permeability in mouse models by an unknown mechanism (LeBerthon et al., 1991), while IL-2 given alone increased vascular permeability in all organs.

Potentially antigenic or otherwise targetable proteins expressed in tumor-associated vasculature (Baillie et al., 1995) and stroma (Dvorak et al., 1991; Rettig et al., 1992) include cellular adhesion molecules (Brooks, 1996; Griffioen, 1997) and receptors for growth factors (Martini-Baron & Marme, 1995). These provide alternative or additional targets for therapy that seeks to destroy tumors by starving them of nutrients and oxygen (Folkman, 1996; Olson et al., 1997; Thorpe & Derbyshire, 1997). Progress has been made in the elucidation of the genetic and environmental factors that control tumor angiogenesis (Fan et al., 1995), which is especially important in animal model systems using xenografted tumors (Damore & Shima, 1996). For example, in one study looking at in vivo model systems for vascular targeting, human tumor xenografts in mice were shown to promote vasculature that expressed mouse, not human, antigenic CD31 adhesion molecules (Lehr et al., 1997). A mouse model system for testing approaches to vascular targeting has been developed in which tumors that secrete interferon-γ induce tumor blood vessel expression of antigenic major histocompatibility complex Class II, whereas normal vasculature expressed Class I (Burrows et al., 1992).

Tumor blood vessels use vascular endothelial growth factor (VEGF) as a survival factor because of constant and extensive remodeling. Normal vasculature, on the other hand, does not require VEGF following embryonic development (Plate et al., 1994). Withdrawal of VEGF leads to apoptotic death of tumor-associated vascular endothelial cells, while overproduction of VEGF leads to hyper-vascularized tumors that are less necrotic (Benjamin & Keshet, 1997; Yuan et al., 1996). VEGF may also be at least partly responsible for enhanced tumor vascular permeability (Roberts & Palade, 1997; Wang et al., 1996). VEGF has been used as a targeting vehicle for truncated diphtheria toxin (Olson et al., 1997). A chemically linked conjugate caused delayed growth of solid tumors in athymic mice. Histological analysis showed tumor necrosis originating from vascular injury and no effect on well-vascularized, normal tissues. In another novel approach to vascular targeting, an experimentally induced Ag in tumor vasculature of large xenografted neuroblastomas in mice was targeted with truncated human tissue factor through a bi-specific Ab (Huang, X. et al., 1997). Blood clots readily formed in tumor blood vessels, leading to 38% partial regressions, while thrombolytic activity in normal vasculature was limited.

2.4. Aspects of tumor metabolism

Deficiencies in tumor-associated angiogenesis can leave large sections (up to 80%; Leith et al., 1991) of sizable tumors without adequate vascularization. The resulting lack of oxygen and other nutrients forces cells to produce energy by glycolysis, leading to a buildup of acidic by-products (Brown, 1997; Helmlinger et al., 1997b). To maintain near-normal cytosolic pH, cells actively export protons (Boyer & Tannock, 1992) so that the extracellular space becomes acidified by an average of 0.5 pH units (Stubbs et al., 1994; Yamagata & Tannock, 1996). In addition, it is thought that some tumors cause a decrease in extracullular pH to allow secreted lysosomal proteases to retain activity for basement membrane digestion as one of the initial steps in metastasis (Montcourrier et al., 1997).

Preclinical approaches have been reported that aim to exploit this pH gradient using bilayer membrane-active agents (Boyer et al., 1993; Karuri et al., 1993) or drugs that act on the Na^+/H^+ exchanger (Hasuda et al., 1994; Maidorn et al., 1993; Yamagata & Tannock, 1996) to kill tumor cells by defeating active proton transport and acidifying the cytosol. These agents are attractive in that they show selectivity in cell killing at low pH (Tannock et al., 1995) against cancer cell populations that have been shown to be genetically more unstable than parental tumor cell lines (Reynolds et al., 1996), and are potentially more metastatic once revascularized (Cuvier et al., 1997; Young & Hill, 1990). Mild acidity within tumor tissue has been proposed to contribute to the selective localization of porphyrinic photosensitizers in photodynamic therapy (Pottier & Kennedy, 1990).

Tumor cells have been shown to be heterogeneous in their ability to survive under hypoxic conditions by down-regulating energy consumption (Skoyum et al., 1997) and up-regulating enzymes such as mitochondrial hexokinase that allow them to optimize energy metabolism (Oudard et al., 1997). Cells that express the bcl-2 protein have been shown to be able to overcome the apoptotic response that normally accompanies hypoxic ATP depletion (Garland &

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