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Targeting the EGF receptor in breast cancer treatment

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Summary

Immunotoxins are a relatively new class of cytotoxic agents consisting of a catalytic toxin linked to an appropriate targeting ligand. The ligand directs the toxin to the surface of a tumor cell, whereupon the toxin enters the cell and catalytically inactivates the ribosome, thus disrupting protein synthesis and effecting cell death. Monoclonal antibodies (or their fragments) have been most commonly used to carry chemically conjugated toxins to proteins or antigens overexposed on the tumor cell surface, but specific ligands for tumor cell surface receptors could also provide effective targeting.

The receptor for epidermal growth factor (EGFR) is overexpressed primarily in poor prognosis breast cancers that do not respond well to traditional therapies. Because EGFR is frequently overexpressed in breast cancer tissue and is associated with a poor prognosis, it is an attractive target for antitumor therapy.

$DAB_{389}EGF$ is an EGFR specific fusion toxin produced with recombinant DNA techniques consisting of sequences for the enzymatically active and membrane translocation domains of diphtheria toxin plus sequences for human epidermal growth factor. $DAB_{389}EGF$ is a potent, EGFR specific, cytotoxic agent which rapidly inhibits protein synthesis by a mechanism of action similar to that of diphtheria itself. Preclinical studies in the laboratory and in animals now suggest the feasibility of investigating such an agent in the targeted therapy of patients with human breast cancer.

Introduction

In the last three decades, significant advances have been made in identifying subgroups of women who require systemic therapy for breast cancer, as well as in the development of treatment programs designed to address systemic disease. However, therapeutic results with chemotherapy and endocrine therapy have plateaued in recent years. More aggressive programs with chemotherapy have led to benefits which occur in conjunction with substantial toxicity, since most chemotherapeutic agents are not specifically cytotoxic to malignant cells. The narrow therapeutic index and unpredictable development of tumor cell resistance to chemotherapy drugs, as well as the poor outcome of patients who relapse following chemotherapy programs, underscore the need for new treatment modalities. Extensive resources have been directed to the identification of new drugs active in breast cancer. Advances have been based upon empiric observations, be-

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cause preliminary studies could predict neither toxicity nor efficacy of new agents. Another approach being investigated is the use of autologous hematopoietic progenitor cell rescue to allow dose-escalation of chemotherapy in women with breast cancer. The results with intensive therapy are promising, but the expense and toxicity inherent in this strategy may limit broad application.

Immunotoxins are a relatively new class of cytotoxic agents consisting of a catalytic toxin linked to an appropriate targeting ligand. The ligand directs the toxin to the surface of a tumor cell, whereupon the toxin enters the cell and catalytically inactivates the ribosome, thus disrupting protein synthesis and effecting cell death. Monoclonal antibodies (or their fragments) have been most commonly used to carry chemically conjugated toxins to proteins or antigens overexposed on the tumor cell surface [1-3]. More recently, a protein hormone has been successfully used in the targeting ligand [4,5].

Certain characteristics are important to consider in the rational design and development of cytotoxic cancer therapy. First, the mechanism of action must be substantially different from available anti-cancer agents. By gaining entry into a cancer cell through binding of a specific antigen or receptor and effecting cytotoxicity through inhibition of protein synthesis, immunotoxins may be active against cells that display a phenotype of chemotherapy drug resistance. Second, immunotoxins can potentially address the systemic dissemination of human breast cancer. These agents do not require activation into cytotoxic metabolites prior to delivery to the target tumor population, so that it should be possible to use them intravenously to treat systemic disease and in defined compartments such as intrathecally, intraperitoneally, or intrapleurally, to treat regional disease.

Selecting the target

Human breast cancer is a tumor in which the cell surface expresses a panoply of hormone receptor molecules, sometimes at very high levels compared to normal tissue, suggesting that investigation of targeted therapeutic approaches taking advantage of these receptors is warranted. Epidermal growth factor receptor (EGFR) mediates the effects of at least two hormones, EGF and TGFα. Both of these hormones function in normal breast growth and development, and may contribute to growth regulation of breast cancer [6-10]. EGFR is expressed in normal mammary gland and levels fluctuate with different stages of glandular development [11]. Binding of ligand to the extracellular domain of the EGFR activates the receptor tyrosine kinase, which causes autophosphorylation of the receptor as well as phosphorylation of other substrates, eventually leading to increased cell proliferation [12]. During this process the ligand-receptor complex is internalized into intracellular compartments where it is degraded. This provides a convenient mode of entry into the cell for a cellular poison that is linked to the ligand itself.

EGFR is expressed in most human breast cancer cell lines to a variable degree [9-13]. Blockade of the EGFR using monoclonal antibodies inhibits EGF and TGFα induced growth [9]. Antibody blockade of the EGFR, or inhibition of EGFR tyrosine kinase activity using a tyrosine kinase inhibitor with some selectivity for EGFR, inhibits *in vitro* as well as *in vivo* growth of a human breast cancer cell line in nude mice without toxicity to the mouse [9,14]. Although some normal tissues also express EGFR, these data suggest that treatments directed at EGFR may have some selectivity for the tumor compared to normal cells, perhaps due to greater EGFR content in the tumor tissue.

Breast cancer tissue from patients also expresses EGFR. Some studies have shown detectable levels of EGFR in 40-50% of breast specimens [10], while other studies using techniques to first dissociate endogenous bound ligand prior to receptor assay found that nearly all human breast cancers express EGFR, sometimes at very high concentrations [15]. EGFR expression has been shown to inversely correlate with poor patient

Table 1. Sensitivity of human breast cancer cell lines to $DAB_{389}EGF$

Cell line	Tissue type	ID_{50} (M)	EGFR/cell
A549	lung adenocarcinoma	$4x10^{-11}$	$5.4x10^4$
SK-BR-3	breast carcinoma	$8x10^{-11}$	$8.7x10^4$
ZR-75-1	breast carcinoma	$1.0x10^{-11}$	$3.3x10^4$
MCF-7	breast carcinoma	$>3.4x10^{-9}$	$8.0x10^2$

Adapted from [23]

prognosis and the lack of response to endocrine therapy, presumably by providing such tumors with a growth advantage [10,16]. A high percentage of breast cancer metastases overexpress EGFR compared to primary tumors. Thus, EGFR overexpression occurs primarily in poor prognosis tumors that do not respond to traditional hormone therapies. Because EGFR is frequently overexpressed in breast cancer tissue and is associated with a poor prognosis, it is an attractive target for antitumor therapy.

Designing the immunotoxin

A number of catalytic protein toxins which intoxicate cells by inhibiting protein synthesis have been used in designing immunotoxins [1,2]. Toxins tested in clinical trials include the plant toxins ricin toxin A chain (RTA), saporin and pokeweed antiviral protein (PAP), and recombinant bacterial toxins including genetically modified pseudomonas exotoxin (PE) and diphtheria toxin (DT). All are extremely potent.

Toxins such as PE and DT have three separate domains responsible for target cell binding, enhancement of translocation into the interior of the cell, and inhibition of elongation factor-2 with cessation of protein synthesis [2]. Ricin has two polypeptide chains which bind the toxin to sugars on the cell surface (B chain) and enzymatically inactivate the ribosome (A chain). Immunotoxins constructed with ricin must therefore have the B chain removed or blocked to avoid non-specific toxicities. The ribosome-inactivating proteins saporin and PAP do not have a B chain but have the enzymatic properties of RTA and can be directly conjugated to produce selective immunotoxins. No toxin has demonstrated a clear advantage over another in clinical trials, but the most experience has been gained with RTA-based immunotoxins. These have demonstrated vascular leak syndrome as the dose-limiting toxicity. In contrast, blocked ricin B chain, PE, and to a lesser degree DT, have induced hepatotoxicity. It does not appear that one toxin is more immunogenic than another. Antibody responses to both ligand and toxin have been reported in all clinical trials to date. Theoretically only an antibody response that interferes with target binding or neutralizes enzymatic activity of the toxin should be of concern. Limited observations from phase I trials support this theory [4,17].

The experience in clinical trials of immunotoxins in cancer has recently been reviewed [1-3]. Most trials have employed monoclonal antibodies (normally linked to RTA or PE) (Table 1). Our studies and others demonstrate several considerations important in the design of targeted therapy trials. First, immuntoxins have been demonstrated to localize to a target tissue, even in patients with solid tumors such as melanoma and colon cancer [18,19]. The heterogeneous nature of the histology and microvasculature of solid tumors is nevertheless problematic because of potentially limited penetration of immunotoxins. The relative accessibility of the target tumor cell may by itself account for the greater success of immunotoxin therapies in hematologic over solid neoplasms. Perhaps even more important is the restricted nature of the expression of certain tumor-associated antigens in hematologic malignancies. Antibodies selected for use in preparing immunotoxins ideally should react with antigens

present only in tumor cells. This level of specificity has been difficult to achieve. The problem has been compounded by the lack of models to assure a thorough preclinical screen of toxicity, which unfortunately led to severe, unanticipated toxicities in early trials [2,20]. On the other hand, certain immunotoxins do seem to be more toxic to the patient's malignant cells than to normal cells expressing the same antigen [17]. Finally, the problems of developing immune response to the immunotoxin have been minimal. Despite the problems, responses and even durable complete remissions have been documented in patients with chemotherapy-resistant malignancies, including solid tumors, with acceptable toxicity [21,22].

The experience with immunotoxins collectively underscores certain considerations in the selection of a target for therapy and in the design of a therapeutic agent. The target must be predominantly expressed on the tumor cell population and must be accessible to the targeted agent. The target must further be capable of internalizing the agent in a way that will allow the toxin access to the cytoplasm. The agent must not be subject to rapid non-specific clearance, and should be extremely potent. Finally, the immunotoxin should be small enough to gain access to tumor cells outside the circulation, and should not engender an immune response.

Ligand fusion toxins

The targeted approach we are presently pursuing has the potential for selectively programming malignant cell populations for death by delivering potent cytotoxins to specific cellular receptors by using a ligand coupled to a toxin. For this strategy to be successful, the targeted receptor must be overexpressed on the cancer cell as compared to normal cells. Further, the receptor should ideally bind a ligand that is critical to the tumorigenesis or continued proliferation of the tumor. The application of recombinant DNA technologies to this problem has allowed the creation of ligands from toxins by genetically linking IL-2, TGFα, EGF, and MSH to either diphtheria toxin or *Pseudomonas* exotoxin [1].

$DAB_{389}EGF$ is an EGFR-specific fusion toxin produced with recombinant DNA techniques by expression of a fusion gene in *E. coli* [23]. The fusion gene consists of nucleotide sequences for the enzymatically active and membrane translocation domains of diphtheria toxin (DT) and sequences for human epidermal growth factor (EGF). $DAB_{389}EGF$ has a molecular weight of 48.5 kDa, enhancing the chances for tumor penetration because of its small size relative to conventional immunotoxins whose molecular weights range above 200 kDa. Because the sequences coding for the hydrophobic portion of DT are preserved along with the ribosylating activity, the resulting fusion toxin combines the potent cytotoxicity of DT with the target cell specificity of EGF.

$DAB_{389}EGF$ is a potent, EGFR-specific, cytotoxic agent which rapidly inhibits protein synthesis by a mechanism of action similar to that of DT itself. When A431 cells, which contain high levels of EGFR, were examined for sensitivity to this agent, the IC_{50} was consistently shown to be only 2 pM [23]. This intoxication is specific for EGFR-expressing cells, since cell lines with relatively few receptors are not affected (Table 2). Additionally, the cytotoxicity of $DAB_{389}EGF$ is inhibited in a dose-dependent fashion by specific competitors of EGF binding. Since some normal tissues also express some EGFR, the therapeutic window may be limited by receptor content. But tumor and normal tissue cell lines expressing little or no EGFR are relatively resistant to the inhibiting effect of $DAB_{389}EGF$ (IC_{50} > 1nM). The 2-4 log disparity in the IC_{50} of sensitive and insensitive cell lines is a promising indicator that a therapeutic window will be observed in the clinic. The cytotoxicity of $DAB_{389}EGF$ as measured by protein synthesis assays does correlate with inhibition of colony growth of human tumor cells. As little as 1 fM of this agent completely ablated the ability of the human squamous cell carcinoma cell line Fa Du to form colonies.

Table 2. Clinical trials with immunotoxins

Disease	Agent	Toxicity	Immune	Tumor response	Ref
Melanoma	Xoma-mel	VLS, myalgia	30/34	1/85 CR, 1/85 PR	19
Breast cancer	260F9-RTA	VLS, neuro, myal	8/9	1/9 PR	20
Colon cancer	Anti-GP72-RTA	VLS, aphasia	16/17	5/16 mixed	18
B-CLL	H65-RTA	VLS, rhabdo	1/11	2/11 PR	25
	T101-RTA	Fever	1/4	0	26
CTCL	H65-RTA	VLS	10/12	4/14 PR	17
Ovarian cancer	OVB3-PE	Neuro	12/16	0	27
	TFR-RTA	Neuro	—	0	28
NHL	Blocked-B4	VLS-hepatic, constitutional	14/25	1 CR, 2 PR	21
	Anti-CD22	VLS, aphasia	4/15	5/14 PR	22

VLS = vascular leak syndrome; CR = complete remission; PR = partial remission

Animal studies

EGFR-expressing tumors have also been shown to be selectively inhibited in an *in vivo* tumor model [24]. Mice were injected with 2 x 10^6 EGFR-expressing tumor cells and treated with DAB_{389}-EGF. Tumor growth was inhibited at doses of the agent that were not associated with toxicity.

Related results in clinical trials

DAB_{389}EGF is just beginning clinical trials in breast cancer patients. But the results my be predicted in part by the experience with two ligand fusion toxins directed to the receptor for interleukin-2. The high affinity receptor for IL-2 is constitutively expressed by the tumors of some patients with certain hematologic malignancies such as non-Hodgkin's lymphoma, Hodgkin's disease, hairy cell leukemia, cutaneous T-cell lymphoma, and the HTLV-I associated T-cell lymphoma. It is expressed normally only on some monocytes and lymphocytes undergoing activation. Both DAB_{486}IL-2 and DAB_{389}IL-2 are recombinant fusion toxins in which the native receptor-binding domain of the toxin has been replaced with human IL-2. Both thus selectively bind and intoxicate cells bearing the high affinity receptor for IL-2, although DAB_{389}IL-2 is about 10-fold more potent. In the first clinical trial of a genetically engineered fusion toxin, we treated 18 patients with 10 daily intravenous bolus infusions of DAB_{486}IL-2. A diverse group of patients with chemotherapy-refractory hematologic malignancies was entered into this trial. Despite the median age of 59 years, DAB_{486}IL-2 was well tolerated as outpatient therapy by this older group of patients. The study group had received an average of 5 different therapeutic regimens prior to study entry, with patients often unable to receive additional therapy due to compromised organ function.

The patients in this study were required to have hematologic malignancies that expressed the IL-2 receptor, as demonstrated by immunostaining with an antibody directed toward the p55 subunit of the IL-2 receptor. DAB_{486}IL-2 was well tolerated at all dose levels, and the patients could receive multiple courses every four weeks. Some patients with antibodies experienced fever, chills, or chest tightness, but no patient discontinued treatment because of these symptoms.

Objective responses were seen in 6 patients, with 1 complete and 2 partial remissions. One patient with a chemotherapy-resistant non-Hodgkin's lymphoma remains in an unmaintained complete remission three years past treatment. The presence of antibodies did not preclude the patients from experiencing an anti-tumor res-

ponse, since 4 of 6 patients with an antitumor effect did have detectable antibodies.

From this initial experience we identified two areas requiring further investigation [5]. In a second phase I trial, we examined the importance of schedule in maintaining prolonged serum levels, and we attempted to improve the assay we were using to detect p55 in clinical specimens. From this additional experience three conclusions were made: 1) Prolonging the infusion can result in achieving target serum levels for a defined period; 2) p55 subunit expression appears to be necessary to achieve a tumor response; 3) A threshold dosage of the agent must be delivered to cause tumor response.

Conclusion

Exploration of $DAB_{389}EGF$ in a phase I trial in patients with EGFR expressing tumors will allow us to establish the feasibility of this approach in breast cancer. Unlike the experience in hematologic malignancies, problems associated with tumor specificity and access must be carefully addressed. However, the attraction of being able to preferentially target malignant breast cells based upon our understanding of their specific cell biology, in this case their excess of EGF receptors, is substantial.

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