Recombinant Immunotoxins in Targeted Cancer Cell Therapy

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Targeted cancer therapy in general and immunotherapy in particular combines rational drug design with the progress in understanding cancer biology. This approach takes advantage of our recent knowledge of the mechanisms by which normal cells are transformed into cancer cells, thus using the special properties of cancer cells to device novel therapeutic strategies.

Recombinant immunotoxins are excellent examples of such processes, combining the knowledge of antigen expression by cancer cells with the enormous developments in recombinant DNA technology and antibody engineering.

Recombinant immunotoxins are composed of a very potent protein toxin fused to a targeting moiety such as a recombinant antibody fragment or growth factor. These molecules bind to surface antigens specific for cancer cells and kill the target cells by catalytic inhibition of protein synthesis.

Recombinant immunotoxins are developed for solid tumors and hematological malignancies and have been characterized intensively for their biological activity *in vitro* on cultured tumor cell lines as well as *in vivo* in animal models of human tumor xenografts.

The excellent *in vitro* and *in vivo* activities of recombinant immunotoxins have lead to their preclinical development and to the initiation of clinical trail protocols. Recent trail

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results have demonstrated potent clinical efficacy in patients with malignant diseases that are refractory to traditional modalities of cancer treatment: surgery, radiation therapy, and chemotherapy.

The results demonstrate that such strategies can be developed into a separate modality of cancer treatment with the basic rationale of specifically targeting cancer cells on the basis of their unique surface markers. Efforts are now being made to improve the current molecules and to develop new agents with better clinical efficacy. This can be achieved by development of novel targeting moieties with improved specificity that will reduce toxicity to normal tissues.

In this review, the design, construction, characterization, and applications of recombinant immunotoxins are described. Results of recent clinical trails are presented, and future directions for development of recombinant immunotoxins as a new modality for cancer treatment are discussed.

I. INTRODUCTION

The rapid progress in understanding the molecular biology of cancer cells has made a large impact on the design and development of novel therapeutic strategies. These are developed because treatment of cancer by chemotherapy is limited by a number of factors and usually fails in patients whose malignant cells are not sufficiently different from normal cells in their growth and metabolism. Other limiting factors are the low therapeutic index of most chemotherapeutic agents, the emergence of drug-resistant populations, tumor heterogeneity, and the presence of metastatic disease. The concept of targeted cancer therapy is thus an important means to improve the therapeutic potential of anticancer agents and lead to the development of novel approaches such as immunotherapy.

The approach of cancer immunotherapy and targeted cancer therapy combines rational drug design with the progress in understanding cancer biology (1–4). This approach takes advantage of some special properties of cancer cells: many of them contain mutant or overexpressed oncogenes on their surface, and these proteins are attractive antigens for targeted therapy. The first cell-surface receptor to be linked to cancer was the EGF receptor, which is present in lung, brain, kidney, bladder, breast, and ovarian cancer (5, 6). Several other members of the EGF family of receptors, the erbB2, erbB3, and erbB4 receptors, appear to be abundant on tumors of breast and ovary and erbB2, for example, is the target for Phase I and II immunotherapy clinical trails (7, 8).

Other promising candidates for targeted therapy are differentiation antigens that are expressed on the surface of mature cells but not on the immature stem cells.

The most widely studied examples of differentiation antigens which are currently being used for targeted therapy are expressed by hematopoietic

malignancies and include CD19, CD20, and CD22 on B-cell lymphomas and leukemias and the IL-2 receptor on T-cell leukemias (9–11). Differentiation antigens have also been found on ovarian, breast, and prostate cancer (12–14).

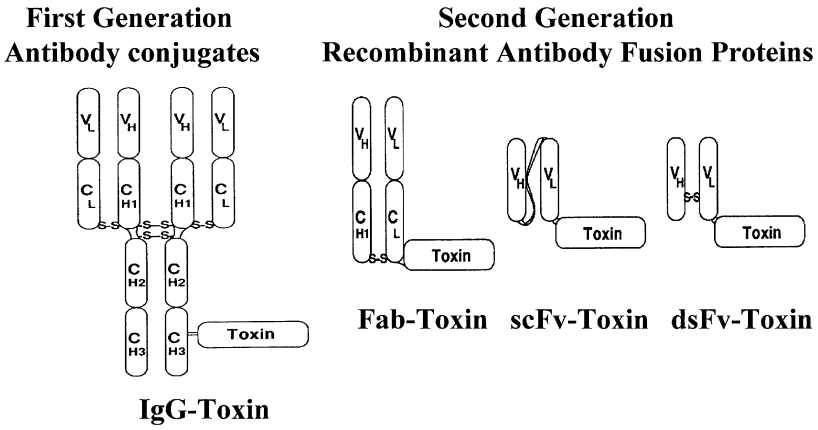
Another class of antigens, termed tumor-associated antigens (TAA), are molecules which are tightly bound to the surface of cancer cells and are associated with the transformed cancer cells. An example is the carbohydrate antigen Lewis Y, which is found in many types of solid tumors (15). Another class of TAAs are cancer peptides that are presented by class I MHC molecules on the surface of tumor cells (16, 17).

It should be possible to use these molecular cell-surface markers as targets to eliminate the cancer cells while sparing the normal cells. For this approach to be successful, we must generate a targeting moiety which will bind very specifically the antigen or receptor expressed on the cancer cell surface and arm this targeting moiety with an effector cytotoxic moiety. The targeting moiety can be a specific antibody directed toward the cancer antigen or a ligand for specific overexpressed receptor. The cytotoxic arm can be a radioisotope, a cytotoxic drug, or a toxin. One strategy to achieve this is to arm antibodies that target cancer cells with powerful toxins which can originate from both plants and bacteria. The molecules generated are termed recombinant immunotoxins.

The goal of immunotoxin therapy is to target a very potent cytotoxic agent to cell surface molecules which will internalize the cytotoxic agent and result in cell death. Developing this type of therapy has attracted much interest in the past years. Since immunotoxins differ greatly from chemotherapy in their mode of action and toxicity profile, it is hoped that immunotoxins will have the potential to improve the systemic treatment of tumors incurable with existing modes of therapy.

As shown in Fig. 1, immunotoxins can be divided into two groups: chemical conjugates (or first-generation immunotoxins) and second-generation (or recombinant immunotoxins). They both contain toxins that have their cell-binding domains either mutated or deleted to prevent them from binding to normal cells, and that are either fused or chemically conjugated to a ligand or an antibody specific for cancer cells (Table I).

First-generation immunotoxins, composed of whole antibodies chemically conjugated to toxins, demonstrated the feasibility of this concept. Cancer cells cultured *in vitro* could be killed under conditions in which the immunotoxin demonstrated low toxicity toward cultured normal cells. Clinical trials with these agents had some success; however, they also revealed several problems, such as nonspecific toxicity toward some normal cells, difficulties in production, and, particularly for the treatment of solid tumors, poor tumor penetration owing to their large size.



MW:	**200 kDA**	**90 kDA**	**66 kDA**	**64 kDA**
Composition:	**Heterogeneous**	**----------**	**Homogeneous**	**----------**
IC_{50} in culture:	**3 ng/ml**		**1.5 ng/ml**	**1.5 ng/ml**
Dose for CR:	**0.75 mg/kg**		**0.063 mg/kg**	**0.075 mg/kg**
T½ in circulation:	**8 h**		**20 min**	**23 min**
Tumor penetration:	**Fair**		**Good**	**Good**

Fig. 1 Immunotoxins for targeted cancer therapy. First-generation immunotoxins are whole monoclonal antibodies to which the toxin is chemically conjugated. Second-generation immunotoxins made by recombinant DNA technology by fusing recombinant antibody fragments to the toxin (usually a truncated or mutated form of the toxin).

Three types of recombinant antibody fragments are used as the targeting moiety in recombinant immunotoxins. Fabs are composed of the light-chain and the heavy-chain Fd fragments (VH and CH1), connected to each other via the interchain disulfide bond between CL and CH1. ScFv fragments are stabilized by a peptide linker which connects the carboxyl terminus of VH or VL with the amino terminus of the other domain. The VH and VL heterodimer in dsFv is stabilized by engineering a disulfide bond between the two domains. The biochemical and biological properties described in the figure are depicted for B3-lysPE38 (LMB-1) (89) (a first-generation antibody–PE chemical conjugate), B3(Fv)-PE38 (LMB-7) (67) (second-generation recombinant scFv-immunotoxin for a scFv-immunotoxin), and B3(dsFv)-PE38 (LMB-9) (74) (for a second-generation recombinant dsFv-immunotoxin).

Second-generation immunotoxins have overcome many of these problems. Progress in the elucidation of the toxins' structure and function, combined with the techniques of protein engineering, facilitated the design and construction of recombinant molecules with a higher specificity for cancer cells and reduced toxicity to normal cells. At the same time, advances in recombinant DNA technology and antibody engineering enabled the generation of small antibody fragments. Thus, it was possible to decrease the size of immunotoxins significantly and to improve their tumor-penetration potential *in vivo*. The development of advanced methods of recombinant-protein

Table I Examples of Recombinant Immunotoxins against Cancer

Immunotoxin	Antigen	Toxin	Cancer	Clinical trail	References
Anti CD7-dgA	CD7	Ricin	Non-Hodgkin's lymphoma	Phase I	156
DAB_{389}-IL2	IL-2R	DT	T-cell lymphoma, Hodgkin's disease	Phase III	122, 125
Anti-Tac (Fv)-PE38 (LMB-2)	CD25	PE	B and T lymphoma, leukemias	Phase I	66,106
DT-Anti-Tac(Fv)	CD25	DT	Leukemias, lymphoma	—	78
RFB4(dsFv)-PE38	CD22	PE	B leukemias	Phase I	76
Di-dgA-RFB4	CD22	Ricin	Leukemias, non-Hodgkin's lymphoma	—	157
B3-lysPE38 (LMB-1)	Lewis Y	PE	Carcinomas	Phase I	89
B3(Fv)-PE38 (LMB-7)	Lewis Y	PE	Carcinomas	Phase I	67
B3(dsFv)-PE38 (LMB-9)	Lewis Y	PE	Carcinoma	Phase I	74
BR96(sFv)-PE40	Lewis Y	PE	Carcinoma	—	95
e23(Fv)-PE38	erbB2/HER2	PE	Breast cancer	Phase I	68
FRP5(scFv)ETA	erbB2/HER2	PE	Breast cancer	—	96
Tf-CRM107	Transferrin-R	DT	Glioma	Phase I	103
HB21(Fv)-PE40	Transferrin-R	PE	Various	—	55
MR1(Fv)-PE38	Mutant EGF-R	PE	Liver, brain tumors	—	99
SSI(Fv)-PE38	Mesothelin	PE	Ovarian cancer	—	100

production enabled the large-scale production of recombinant immunotoxins of high purity and quality for clinical use in sufficient quantities to perform clinical trials.

Another strategy to target cancer cells is to construct chimeric toxins in which the engineered truncated portion of the toxin (PE or DT) gene is fused to cDNA encoding growth factors or cytokines. These include transforming growth factor (TGF)-α (18), insuline-like growth factor (IGF)-1 (19), acidic and basic fibroblast growth factor (FGF) (20), IL2 (21), IL4 (22), and IL6 (23). These recombinant toxins (oncotoxins) are designed to target specific tumor cells that overexpress these receptors.

This review will summarize knowledge of the design and application of second-generation recombinant Fv immunotoxins, which utilize recombinant antibody fragments as the targeting moiety, in the treatment of cancer,

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