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Review

Antibody-cytokine fusion proteins for improving efficacy and safety of cancer therapy

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ABSTRACT

Cytokines are key players in the regulation of immune responses both in physiological and pathological states. A number of cytokines have been evaluated in clinical trials and shown promising results in the treatment of different malignancies. Despite this, the clinical application of these molecules may be plagued by undesirable side effects The development of recombinant antibody–cytokine fusion proteins, which offer a means for target delivery of cytokines toward the tumor site, has significantly improved the therapeutic index of these immunomodulatory molecules. Selective tumor localization is provided by the monoclonal antibody component of the fusion protein that binds to the molecules present on the surface of tumor cells or accumulated preferentially in the diseased site. In this manner, the cytokine element is specifically located at the tumor site and can stimulate immune cells with appropriate cytokine receptors. Over the recent years, several antibody–cytokine fusion proteins have been developed with the capacity to target a wide variety of cancers whose application, in some cases, has led to complete rejection of the tumor. These findings support the notion that antibody–cytokine fusion proteins represent huge potential for cancer therapy. This review presents an overview of the advances made in the field of targeted cytokine delivery, which is made possible by genetically engineering antibody–cytokine fusion proteins.

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1. Introduction

The immune system plays important roles in battle with different diseases including cancer. In addition to antibodies that are capable of identifying cancer cells and redirecting immune

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response toward cancer cells through processes such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), tumor immunotherapy also uses substances called cytokines, which can act as immunomodulatory agents [1]. Cytokines can function locally (via autocrine and/or paracrine pathways), resulting in proliferative responses, differentiation, chemotaxis, inflammatory reactions, innate and adaptive immunity, and cell death [2]. Cytokines are mediators and regulators of the innate and adaptive immune system, and can also act as hematopoietic growth factors [3,4]. Immunotherapy by using cytokines was first introduced with regard to interferons (IFNs) and interleukin-2 (IL-2) [5,6]. The first cytokines approved for cancer therapy were IFN-α and IL-2 [7,8]. A number of other interferons and interleukins are currently being evaluated in clinical trials [9]. Severe cytotoxicity remains the major limitation for using cytokines in the clinic. Therefore, some strategies have been developed to improve the therapeutic efficacy of these molecules. One of these strategies is connecting cytokines to antibodies, which may lead to targeted cytokine delivery [10,11]. These "antibody-cytokine fusion proteins" merge the specific targeting capability of antibodies with the immunomodulatory functions of cytokines [12–14]. The primary objective of this approach is to concentrate cytokines in the tumor site, enhance the tumoricidal effect of the antibody molecule, and increase the host immune reaction against tumor, while severe toxic effects caused by high amounts of systemic cytokine are prevented [15–18]. Now, most of antibody fusion proteins developed for tumor immunotherapy are antibody–cytokine fusion proteins, which consist of an antibody able to target a tumor-associated antigen and a cytokine able to raise the immune reaction (Table 1). Given that cytokines usually exhibit high systemic toxicity, their antibody-mediated accumulation in tumor allows researchers to administer less effective dosage, which decreases the risk of unfavorable events associated with high cytokine concentrations [19]. Over the recent years, introduction of the concept of tumor immunosurveillance, which refers to the identification and removal of nascent cancer cells by the immune system, has highlighted the vital role as well as the capacity of the body's immune system against tumorigenesis [20]. Directing toward tumor can be achieved by specific antibodies that target tumor-associated antigens. Immunotherapeutic methods make use of monoclonal antibodies with humoral and cellular effector roles, bispecific antibodies, and genetically engineered chimeric antigen receptors (CARs), which retarget T cells toward tumor cells [21–25]. Now, immunotherapeutic methods depend mainly on using immunomodulatory cytokines, agonistic monoclonal antibodies targeted against costimulatory receptors, and antagonistic monoclonal antibodies targeted against coinhibitory receptors [26–28]. The current review will discuss the antibody-cytokine fusion proteins examined to date, with a particular focus on those that have receiving attention in clinical trials.

2. Targeted cytokine delivery

Most cytokines act as soluble factors, which are able to bind to their cognate receptor(s) on the target cell and activate them. Therefore, the use of cytokines for cancer treatment via intravenous route requires their accumulation in the tumor site, which allows them to perform their immunomodulatory or cytotoxic functions [29,30]. It has been suggested that combining cytokine molecules with a targeting moiety leads them to accumulate within tumor and act on the target cells [15,17,31–35]. Sending cytokines to the surrounding area of cancer cells seems, in most conditions, to be adequate for the initiation of a stimulatory action on immune cells. This action can be implemented either on the cell to which the fusion protein (via the antibody molecule) binds (cis-acting), or on neighboring cells (trans-acting). Because of the antibody-mediated attachment to the cell surface, the protein fused to the antibody can mimic membrane-bound cytokines. It has been discovered that transmembrane and soluble cytokines act differentially. For instance, membrane TNF (Tumor Necrosis Factor) activates both TNF receptors (TNFR1 and TNFR2), but soluble TNF (sTNF) only activates TNFR1. Each of these functions can cause different cell selectivities and cellular outcomes [36–38].

3. Antibody–cytokine fusion proteins

The benefit of antibody-mediated targeted delivery has been demonstrated in mouse models by comparing antitumor effects caused by IgG-IL-2 molecules attached to tumor-specific or nonspecific antibodies [39–41]. With antibody–cytokine fusion proteins, accumulation of the cytokine in the tumor site is achieved by the antibody component. The development of specific antibodies has been performed by selecting monoclonal antibodies against tumor-associated antigens such as carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR), etc. In general, antibody-cytokine fusion constructs can be made by fusing cytokines to (i) complete immunoglobulins (Ig fusion), (ii) the Fc fragment of an antibody (Fc fusion), or (iii) antigen-binding fragments such as Fab, single-chain Fv (scFv) or diabodies. IgG

Table 1
Antibody fusion proteins in clinical trials for cancer immunotherapy (see ClinicalTrials.gov).

Format	Format	Cytokine	Antigen	Indication	Stage
hu14.18-(IL-2) (EMD 273063)	IgG-IL-2	IL-2	GD2	Neuroblastoma and melanoma	Phase II
DI-Leu16-(IL-2)	IgG-IL-2	IL-2	CD20	NHL	Phase I/II
L19-(IL-2)	Db-IL-2	IL-2	ED-B	Colon cancer, teratocarcinoma, small-cell-lung cancer and other solid tumors	Phase I/II
F16-IL2[a]	Db-IL-2	IL-2	A1domain of Tenascin C	Breast cancer, lung cancer	Phase Ib/ II
HRS3-scFv-hi-(IL-12)	scFv- IL-12	IL-12	CD30	Hodgkin's lymphoma	In vitro
AS1409 (huBC1-huIL12)	IgG-IL-12	IL-12	ED-B (fibronectin)	Metastatic renal cell carcinoma, metastatic melanoma	Phase I
hu14.18-(GM-CSF)	IgG-(GM-CSF)	GM-CSF	GD2	Neuroblastoma	Phase I
L19-TNFa	scFv-TNF	TNF	ED-B (fibronectin)	Advanced solid tumors	Phase I/II
NHS-IL12	IgG-IL-12	IL-12	DNA	Epithelial Neoplasms, Malignant Epithelial Tumors, Malignant, Malignant Mesenchymal Tumor	Phase I
Anti-CD20 IFNα, (IGN002)	IgG- IFNα	IFNα	CD20	B cell NHL	Phase I

IgG: Immunoglobulin G; ED-B: Extra domain-B of the B-FN isoform of human fibronectin; GD2: Disialoganglioside; NHL: Non-Hodgkin's lymphoma; Db diabody; IL interleukin; scFv: Single-chain Fv; EpCAM: Epithelial cell adhesion molecule; RCC, renal cell carcinoma.
[a] F16-IL2 use in combination with Doxorubicin.