ANTIBODY-CYTOKINE FUSION PROTEINS: PRODUCTION, FUNCTIONALITY AND APPLICATION PROSPECTS IN ONCOLOGY

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In medicine cytokines play an important role as the immune response modulators. However, biologically active drug application in high doses in oncology is followed by a number of unfavorable side effects resulting in treatment cessation. Target therapy enables to increase the efficiency of cytokine usage, and therefore, reduce the drug doses.

The achievements in genetic engineering and biotechnology led to a growing number of new antibody-cytokine fusion proteins. Such hybrids can have the properties of all components and acquire advantages compared to proteins alone. For example, monoclonal antibodies specific to a particular tumor antigen being fused with cytokines (MAb–C) provide accumulation of cytokines in tumor microenvironment, increase antitumor effect of antibodies and enhancement of the immune response against a tumor. MAb–C with various specificity against a number of tumors have been created in the last twenty years. It was shown on animal models that such fusion proteins being accumulated around a tumor are capable to cause the considerable antitumor response, which in some cases results in complete tumor elimination. The present review describes data on existing models of antibody-cytokine fusion proteins, their technology and application prospects in oncology.

Key words: cytokines; antibodies; fusion proteins; antitumor drugs.

Cytokines consider to be the protein nature mediators, mediator molecules, which take part in intercellular signal transmission. Their main role is to simulate an immune response. A cytokine binding with specific receptors on cytoplasmic membrane activates the mechanism of intercellular signal transmission providing the regulation of a number of genes responsible for the synthesis of both: modulators themselves, and other cytokines, as well as the formation and appearance of cytokine receptors on cell surface [1–3].

By now over 100 various cytokines have been discovered and described [4]. Among these are interleukins (IL), interferons (IFN), colony-stimulating factors (CSF), tumor necrosis factor (TNF), growth factors and chemokines. These mediators exhibit diversified activity, which partially cross. Cytokines are rarely formed separately and rarely act singly. Cytokine system response is of complex network nature, when the production of one of them has an effect on the formation or activity of others [5–7].

Many cytokines have direct antitumor activity or are the mediators of antitumor immunity [8, 9]. Systemic high-dose cytokine therapy is frequently accompanied by severe side effects, which make further use of cytokines at recommended dosage impossible [10–12]. Target injection of a drug into a tumor partially solves the problem, since cytokine concentration at injection site decreases rapidly. Moreover, frequently tumor or metastases localization make it impossible to carry out a procedure [13, 14].

Other approach is to use gene therapy [15, 16]. In this case a vector carrying gene responsible for cytokine synthesis is injected. The expected result is systemic immune response against tumor [17, 18]. This technology is highly complicated, takes a long time and is expensive, that limits its application.

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Tumor-specific monoclonal antibodies genetically fused with cytokines (MAb–C) are an alternative option for intratumoral cytokine accumulation in the concentration sufficient to produce significant antitumor effect without systemic toxicity accompanied. Cytokines can have an effect on both the cell that have bond the antibodies with, and neighboring cells. Moreover, due to the binding to cell surface through antibodies, fusion proteins can simulate cytokine transmembrane form. It has been demonstrated that some cytokines exist both dissolved, and being bind to membrane, and their functions differ. These effects are described, for instance for TNF [19].

As well as antibodies provide cytokine delivery to tumor, they also can be used as antitumor agents by blocking the receptors on the surface of tumor cells [20–24]. About 50% of all antibodies on pharmaceutical market are antitumor, and in 2013 about 10 new agents are expected to appear, which are meant for the treatment of different tumors [25].

Currently, a variety of tumor-specific MAb–C containing different cytokines has been developed and is being under trial (at various phases) [26, 27]. This review summarizes the knowledge on existing fusion proteins, their technologies and prospects for application in oncology.

Fusion proteins technology

There are five classes of human immunoglobulins (antibodies): IgG, IgA, IgM, IgD and IgE, which vary in molecule size, charge, amino acid profile, and carbohydrate status. At the same time there is significant heterogeneity within the limits of an each class. The main structural unit of any class immunoglobulin consists of two similar light and two similar heavy chains held together by disulphide bonds (See Fig. *a*). Each light chain consists of one variable (V_L) and one constant domain (C_L), while a heavy chain — of one variable (V_H) and three constant domains (C_H1, C_H2, C_H3).

Variable domains of a heavy and light chain form an immunoglobulin variable region (Fv) providing a specific link with antigen. Antibodies can be split into three fragments using papain protease: two Fab (fragment antigen binding) and one Fc (fragment crystallizable). Fab contains all light chain, V_H-domain and C_H1, Fc — all other C-domains of a heavy chain. The division occurs in a hinged region (HR) — it is a specific part of a polypeptide chain, which is not a part of domains and genetically not related to them.

Classical technique of monoclonal antibodies production is hybrid technology [28, 29]. The idea lies on the fact that myeloma cells are fused with B-lymphocytes secreting specific antibodies after mice being immunized by an appropriate antigen. Hybrid cells (hybridomas) are capable of unrestricted division and synthesis of target antibodies. Such murine antibodies show antitumor effect blocking the receptors on tumor cells and counteracting fluid-phase ligands, as well as inducing apoptosis [30]. However, Fc-region of murine antibodies is not capable of binding to human effector cells completely that limits their therapeutic potential. Moreover, such an agent is immunogenic, and in patients it causes a response against murine antibodies with their further neutralization and degradation [31, 32].

Despite significant target efforts, no adequate approaches have been developed to obtain hybrids based on human cells. Currently, the problem is solved using molecular-genetic approaches [33]. Humanization of murine antibodies is the most common approach. The technique consists in the formation of fusion genetic complexes uniting V-gene of murine monoclonal antibodies and C-genes

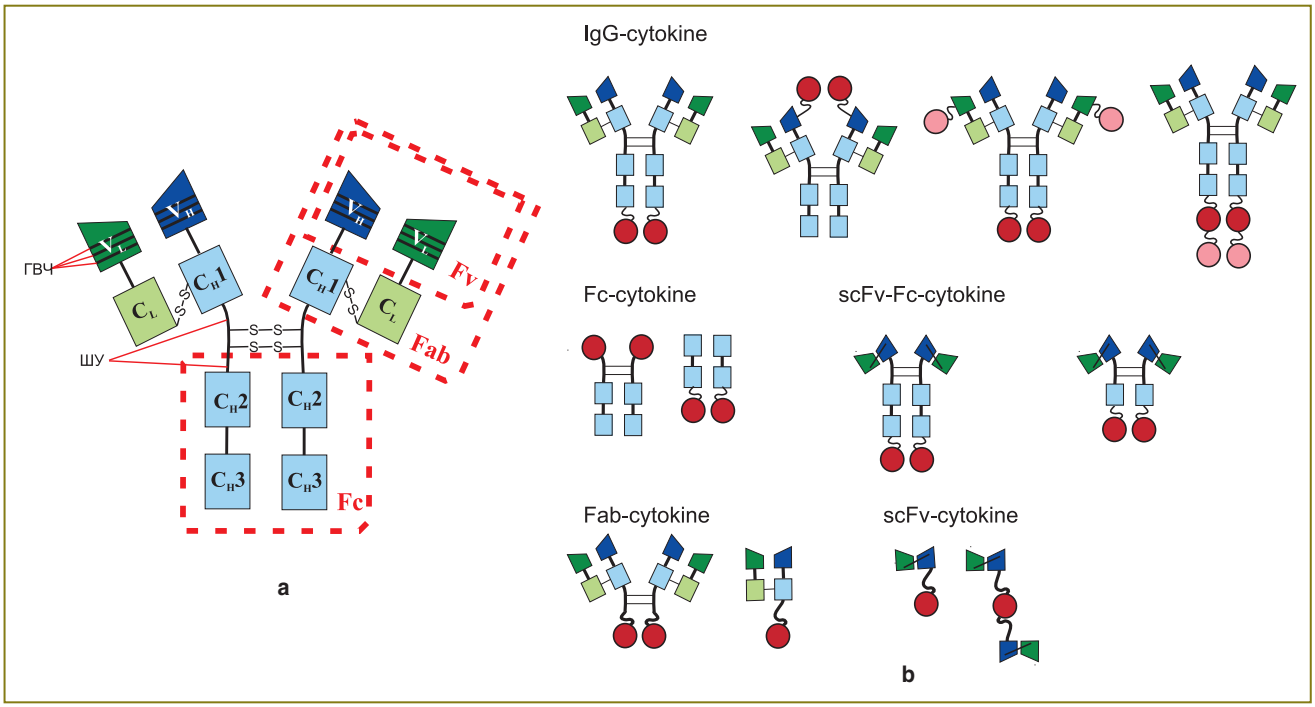

Fig. Schematic diagram of IgG molecular structure (*a*) and examples of existing fusion proteins based on antibodies or mini-antibodies (*b*). The description is given in the text

of human immunoglobulins of a desired isotype. Due to the presence of human constant region, these antibodies have a wide range of biological functions. Rituximab is an example of humanized antibody (Rituxan, MabThera) [34–36]. Rituximab recognizes antigen CD20 on the surface of normal and tumor B-cells and induces cell-mediated and complement-mediated cytotoxicity and apoptosis of these tumor cells. There is the more radical approach consisting in fusing hypervariable regions of murine V-genes with genes encoding framework sequence of human V-gene and human C-genes. Such humanized antibodies have the only part remained after mice — a hypervariable region. Trastuzumab (Herceptin) is an example, these antibodies bind to receptor 2 of human epidermal growth factor (HER2). These antibodies are recommended for breast cancer therapy [37–39].

To exclude immunogenicity there is a need to obtain fully human antibodies. For that purpose there were produced transgenic mice, immunoglobulin genome of which was replaced with human ones (of XenoMouse line, Tc-mice). Such mice produce human polyclonal antibodies [40–42].

An alternative of fully antibodies is mini-antibodies with two chains of a single-chain variable antibody region (scFv), a hinged region and C_H3-domain or C_H2–C_H3-domains [43–44]. Unlike the first ones, they are characterized by higher elimination rate (10 h instead of 3–4 weeks). Among the disadvantages of mini-antibodies there is the loss of some functions including cell-mediated cytotoxicity, which plays a key role in antitumor mechanism of antibody action [45].

Phage display technology can also be used to receive human antibodies [46, 47]. scFv genes are cloned in phage display vector. scFv fragment expressed on bacteriophage surface has a high degree of relationship with a target antigen.

In the past decade there has been developed a number of MAb–C of various structure and functional significance. Both whole antibodies and their fragments can underlie these recombinant proteins (See Fig. *b*). The variety of fusion proteins is provided by cytokines used, among them there are both monomers and homodimers or homotrimers, in addition, there are cytokines formed by different polypeptide chains — heterodimers [4].

Currently, recombinant MAb–C present two main forms: F(ab)2/C secreted by mammal cells, and a single-chain FV/C (micro-antibodies) expressed by *E. coli*. The first one are large molecules, 134–140 kDa, consisting of a pair of light chains and a pair of hybrid heavy chains, each of which includes a variable region C_H1, a hinged region and a cytokine. Micro-antibodies, 42–45 kDa, consist of one variable fragment of a heavy chain, one variable fragment of a light chain, and a cytokine. In the early 90-s there was produced MAb–C, in which a cytokine binds to C-terminus of C3-domain of a heavy chain. The researchers demonstrated that in most cases such molecules retain both antibody (capability to bind to antigen), and cytokine functions. This indicates the feasibility of DNA-technology to produce bifunctional proteins.

The use of mammalian cells as expression system for MAb–C (e.g., newborn hamster cell culture) provides posttranslational human specific modifications that increase biological activity of recombinant immunoglobulins and *in vivo* complex stability [48].

Plants with temporary expression of recombinant proteins can be one more producer of fully antibodies genetically fused with cytokines [49]. Currently, Trastuzumab analog (Trastuzumab, Herceptin) was produced using this technology [50].

Prospects for MAb–C application in oncology

The pharmaceutical market has no MAb–C approved for anticancer therapy. However, a number of pharmaceuticals are under clinical trials (I–II phases). Preliminary trial data indicate extensive prospects to use recombinant MAb–C as anticancer drugs. Due to a growing number of antibody-cytokine combinations (Table 1) it is impossible to cover all existing variants in the present paper. Let us consider the most developed and advanced variants, in which monoclonal antibodies bind to IL-2, IL-12, GM-CSF and TNF.

MAb–IL-2. IL-2 is known as an immunomodulator of cellular and humoral immunity with an extensive therapeutic potential [51, 52]. This cytokine is capable to inhibit tumor growth inducing apoptosis due to its capability to stimulate macrophages and NK-cells and increase the expression of histocompatibility molecules class II [53]. E. Ortiz-Sanchez et al. described the main existing MAb–IL-2 and their functional value [26]. First MAb–IL-2 was developed in the early 90-s. They presented IL-2 molecule bound to C-terminus IgG3 specific to dancyl [54]. IgG3–IL-2 could stimulate proliferation of IL-2-dependent murine T-cells of CTLL-2 line. This fusion protein showed higher (approximately 4 times as high) affinity than recombinant human IL-2, and was more effective at activation of LAK-cells (lymphokine-activated killer). Moreover, IgG3–IL-2 half-life at gastric administration in mice was 7 h that is longer than free IL-2 administration, but shorter than using single IgG3 injection.

There were developed two MAb–IL-2 specific to Id-antigen, one of which included complete IgG1, another — a single chain fragment scFv IgG1 [55]. The researchers showed scFv–IL-2 capacity to bind to antigen to be 30–40 times as low than that of IgG1–IL-2. In addition, scFv–IL-2 is excreted 20 times as fast than IgG1–IL-2. Finally, scFv–IL-2 has no Fc-region necessary to induce antibody-dependent cell-mediated cytotoxicity, and plays a key role in IgG1–IL-2 antitumor activity.

20–30% cases of breast and ovarian cancer show hyperexpression of HER2/neu (erbB2) receptor. The above mentioned Trastuzumab (Herceptin) used in the pharmaceutical market presents monoclonal antibodies to this receptor. Trastuzumab bound to HER2/neu stops cell cycle at G1 phase thus reducing the proliferation of tumor cells. There has been developed anti-HER2/neu IgG3–IL-2 [56]. This fusion protein retains the capability of antibodies to bind to an appropriate receptor, and biological activity similar to recombinant IL-2. There has been studied anti-HER2/neu IgG3–IL-2 effect on murine intestinal tumor cells expressing human HER2/neu. The use of this MAb–C caused significant tumor growth inhibition, while antibodies (anti-HER2/neu IgG3) had no effect.

Table 1

Summary table on existing fusion proteins based on cytokines and antibodies to antigens presented in the table

Cytokines	Antigens, specificity														
	Lymphoma	Depending on an antibody chosen	Breast cancer, ovarian cancer, colon cancer	Ovarian cancer	Hodgkin's lymphoma	Breast cancer, non-Hodgkin's lymphoma, chronic lymphatic leukemia	Melanoma, neuroblastoma, glioblastoma, small-cell carcinoma of lung	Carcinomas (including prostate cancer), breast cancer, ovarian cancer, colon cancer	Gastrointestinal cancer, bile duct carcinoma	Rheumatoid arthrites	Teratocarcinoma, colon cancer, prostate cancer, skin cancer	Breast cancer, colorectal cancer and lung cancer	Non-Hodgkin's lymphoma, chronic lymphatic leukemia	Glioma, colon cancer, pancreatic carcinoma, neuroblastoma, etc.	Adenocarcinoma (epithelial grandular cells), breast cancer
	DNC	Id	HER2/neu	TAG-72	CD30	hMHC II	GD2	EpCAM	CEA	ED-A	ED-B	FAP	CD20	TfR	MUC-1
IL-2	+	+	+		+	+	+ !!	+ !					+		+
IL-6		+													
IL-7										+					
IL-10										+	+				
IL-12			+		+			+	+		+ !				
IL-15							+				+	+			
IL-17															
IFN-α			+							+			+		
IFN-γ				+							+				
GM-CSF		+	+			+	+!	+			+			+	
TRAIL			+					+							
TNF			+	+					+		+!!				
FasL												+	+		

H e r e: GM-CSF — granulocyte macrophage CSF; TRAIL — TNF related apoptosis-inducing ligand; FasL — ligand for Fas membrane molecule; DNC — dancyl chloride (5-(dimethylamino)naphthalene-1-sulphonyl chloride); Id — idiotypic antibodies, analogs or antigen imitators; EpCAM — epithelial cell adhesion molecule; CEA — carcinoembryonic antigen; TfR — transferring receptor; ED-B, ED-A — isoforms of fibronectin; FAP — stromal fibroblast activation protein; ! – phase I clinical trials; !! – phase II clinical trials.

The other MAb–C, anti-erbB2 scFv-Fc–IL-2, was developed on the basis of C_H2–C_H3-domains of human IgG1 combined with murine scFv specific to HER2/neu. This fusion protein also retained both antibody and cytokine activity [56–58] that was confirmed on T-cells of CTLL-2 line, as well as on BALB/c mice with induced ovarian cancer, the cells of which are characterized by high HER2/neu expression. Intravenous injection of scFv-Fc–IL2 protein to mice resulted in tumor growth reduction.

Mucin-1 (MUC-1) is a transmembrane protein naturally expressed on the surface of grandular epithelial cells. In carcinomas there is hyperexpression of this protein on tumor cell surface. Thus, MUC-1 can serve as a marker antigen, and specific antibodies can be used for cytokine target delivery in tumor microenvironment. For this purpose scFv of murine MUC-1-specific antibodies was genetically fused with N-terminus of a hinged region of human IgG1 Fc-region, and IL-2 was fused with C-terminus of Fc-region. The obtained fusion protein retained the capability to bind to MUC-1 expressed by human mammary adenocarcinoma cells, as well as biological activity of IL-2 consisting in its capability to induce the proliferation of $CD25^+$-lymphocytes and activate NK-cells [59].

In Hodgking's lymphoma an increased number of CD30 is secreting on the surface of tumor cells, and antiCD3 antibodies were also used for producing fusion proteins for IL-2 delivery. Recombinant anti-CD30 MAb–IL-2 was produced on the basis of scFv-region of monoclonal anti-CD30 antibody, N-terminus of which was fused with IgG1 hinged region, bound by C-terminus with IL-2. Anti-CD30 MAb–IL-2 was bifunctional, activated T- and NK-cells, induced *in vivo* IFN-γ production. *In vivo* studies supported the suggestion that this fusion protein can be used in specific immune therapy in Hodgking's lymphoma [60].

Characteristic marker of non-Hodgkin's lymphoma is CD20. Fusion anti-CD20 MAb–C were fully humanized murine monoclonal antibodies genetically bound to IL-2. In experiments anti-CD20 MAb–IL-2 induced apoptosis of $CD20^+$ Daudi cells (human lymphoma cell line) and retained their capability to bind to a specific receptor and exhibit antibody-dependent cell-mediated cytotoxicity. The produced fusion protein induced antitumor immune

response in animal model on SCID mice, which were injected with human lymphoma line cells Daudi Burkitt $CD20^+$ intravenously simulating disseminated lymphoma [61].

MAb–IL-12. IL-12 is a heterodimer protein with molecular mass of 75 kDa [62]. Its main producers are monocytes, macrophages, as well as dendritic cells, neutrophils and lymphocytes. IL-12 activates the proliferation and cytotoxic activity of T- and NK-cells. The main effect is the stimulation of IFN-γ production, which can inhibit tumor growth and increase the expression of MHC class I genes. In addition, IL-12 has anti-angiogenic activity due to the regulation of IFN-γ-dependent proteins IP10 and MIG (monokine induced by IFN-γ). These cytokines inhibit chemotaxis of endothelial cells and block their differentiation [63].

IL-12 exhibited potential antitumor activity on various animal models as well as was used for the treatment of viral and bacterial infections [64]. However, in clinical studies IL-12 was shown to be less active, and there were severe side effects [65, 66].

In vivo studies demonstrated that fusion anti-HER2/neu MAb–C(IL-12) retains the functions of IL-12 (heparin-binding activity, the capability to induce IFN-γ secretion) [26] and IgG (capability to bind to human antigen HER2/neu) [67]. A prolonged administration of anti-HER2/neu MAb–IL-12 in mice was also shown to cause a long-term immune response spreading over other antigens [68].

In order to study MAb–IL-12 anti-angiogenic activity there were developed fusion proteins against human fibronectin ED-B domain (an angiogenesis marker secreted by tumor and endothelial cells). *In vitro* studies demonstrated that the obtained recombinant protein possessed IL-12 biological activity and antibody specificity. Anti-ED-B MAb–IL-12 capability was shown to inhibit tumor growth *in vivo* on mice models (colon carcinoma and teratocarcinoma). Moreover, a recombinant protein was localized in tumor microenvironment prior to IL-12. In addition, there were no side effects [69].

The comparison of anti-HER2/neu MAb–IL-12 effect with combined administration of anti-HER2/neu MAb and IL-12, as well as IL-12 monotherapy proved the significance of physical fusion of antibodies with cytokine to increase antitumor activity. The researchers link it to cytokine placement in extracellular matrix of tumor microenvironment [70].

There were also produced anti-antigen CD30 and CEA fusion proteins (See Table 1). *In vitro* studies demonstrated them to retain both cytokine function (the ability to induce secretion by IFN-γ T- and NK-cells), and antibody function (the ability to bind to antigen) [71, 72]. AS1409 is a fusion protein based on humanized antibodies specific to fibronectin and bound to IL-12. Its first clinical trials indicate the approach safety. In this study maximum tolerated dose was used and effectiveness against metastatic melanoma was demonstrated [73].

MAb–GM-CSF. GM-CSF refers to a group of glycoproteins regulating the proliferation and differentiation of hemopoietic cells [74]. The main field of this cytokine application is prevention of neutropenia and neutropenic complications in patients with highly decreased neutrophils in blood after cytostatic chemotherapy due to different tumors [75, 76]. In addition, GM-CSF regulates the expression of MHC class II and antigen-presenting capability of APC (antigen-presenting cells). A wide range of action makes this cytokine be potential medication for adjuvant antitumor immune therapy [77].

Systemic GM-CSF injection is accompanied by a number of side effects: fever, shiver, myalgia, loss of appetite, drowsiness, and ostealgia [78, 79]. In order to reduce negative effects and to increase anti-tumor activity, GM-CSF was genetically bound to antibodies against various tumor antigens (See Table 1).

Transferin (Tf) is a glycoprotein transferring iron ions necessary for cell proliferation. Tf receptor's hyperexpression is observed on several types of cancer cells. GM-CSF fused with anti-TfR antibodies was shown on a mice model to demonstrate antitumor activity resulting in growth reduction of murine metastatic hepatic neuroblastoma (NXS2) and lung metastases of murine colon carcinoma (CT26). The data obtained indicate possible usage of a similar fusion protein to treat patients with malignant tumors characterized by TfR hyperexpression [80].

There were produced chimeric (human/mouse) anti-ganglioside GD2-antobodies. This fusion protein exhibited antigen-dependent cell associated cytotoxicity and complement-dependent cytotoxicity in neuroblastoma cells NMB7 and mononuclear cells isolated from the same patients. *In vivo* studies showed anti-GD2–GM-CSF to be characterized by higher adhesive and degranulating property compared to antibodies or cytokines. Currently, the products based on anti-GD2–GM-CSF and anti-GD2–IL-2 are under clinical trials [81, 82].

Other researchers studied the biological activity of anti-MHC II–GM-CSF. This fusion protein demonstrated the properties of both cytokine (the capability to induce the formation of hemopoietic progenitor cells from bone marrow mononuclear cells), and also antibodies (the capability to bind to tumor cells expressing MHC class II). The studies of biodistribution on a murine xenograft model proved this fusion protein to be capable of selecting specifically human malignant B-cells [83].

Bifunctionality of fusion MAb–C was also demonstrated on anti-ED-B–GM-CSF. This fusion protein exhibited high tumor specificity and capability to reduce significantly tumor growth in experiments on mice 129SvEv with induced teratocarcinoma F9 and adenocarcinoma of colon C51. In addition, an antimetastatic effect was demonstrated on the same models [84].

For the purpose of in-depth study of MAb–C biological activity, effects and mechanisms of action, there were produced murine anti-HER2/neu–GM-CSF [85]. This fusion protein retained the property of cytokine, namely, the ability to stimulate the growth of ABC-31 line myeloid cells, as well as activate murine macrophage cells J774.2 and enhance antibody-dependent cytokine-mediated lysis in tumor cells. On the other hand, anti-HER2/neu–GM-CSF binds to murine tumor cells CT26 expressing HER2/neu receptors on its surface, and enhances anti-HER2/neu immune response. An important point is that this fusion protein causes tumor growth rate reduction on those models, on which the use of

Table 2

Di-cytokine fusion proteins

MAAb–C1C2	Target antigen	Target tumor for fusion protein	Status	References
Anti-EpCAM–humanGM-CSF/IL-2	EpCAM	Colon cancer	Animal model	[97]
Anti-EpCAM–murineGM-CSF/IL-2	EpCAM	Gastric cancer	*In vitro*	[98]
Anti-HER2/neu–IL-12–IgG3–IL-2	HER2/neu	Breast cancer and colon cancer	Animal model	[68, 99]
Anti-HER2/neu–IL-12–IgG3–GM-CSF	HER2/neu	Breast cancer and colon cancer	Animal model	[68, 99]
IL-12–L19–TNF-α	ED-B	Teratocarcinoma	Animal model	[100]
KS–IL-12/IL-2	EpCAM	Lewis lung carcinoma	Animal model	[101]
Anti-CD30–IL-12/IL-2)	CD30	Hodgkin's lymphoma	Animal model	[102]

antibodies only does not provide protection. These findings support once again the potential significance of anti-HER2/neu–GM-CSF for managing patients with HER2/neu-positive tumors.

MAb–TNF. Tumor necrosis factor is an extracellular multifunctional cytokine produced mainly by monocytes and macrophages [86, 87]. TNF induces apoptosis in endothelial cells in new-formed vessels promoting agents to penetrate into tumor mass and causing hemorrhagic tumor necrosis [88]. However, systemic injection of this protein is strictly limited by high toxicity consisting in a syndrome like septic shock resulting in multiple organ failure.

In order to reduce a therapeutic dose of high-activity protein, fusion protein L19–TNF was produced, which should provide TNF target delivery in tumor blood vessels [89]. L19–TNF is anti-B-domain fibronectin L19 antibodies bound to TNF. Preclinical studies on animals demonstrated L19–TNF bifunctionality. In phase I-II of clinical trials there were chosen safe therapeutic doses of the drug. Non-hematological toxicity at such doses was shown to be low, although in rare cases severe myelosuppresion was recorded [90, 91].

Conclusion

To sum up the described data, we can specify the main advantages of applying fusion proteins based on antibodies and cytokines.

1. A recombinant fusion protein is found in tumor microenvironment more quickly than the cytokines alone. The presence of cytokine receptors in normal cells provides competition for fusion protein molecule binding that reduce MAb–C accumulation in the tumor area. Nevertheless, the experiments on animal models and in clinic showed MAb–C concentration in tumor is higher than via systemic injection of free antibodies. MAb–C being concentrated mainly in tumor has less toxic effect on normal organs and tissues. Due to its relationship with specific antigen, cytokine washout period increasesand therapeutic effect is prolonged.

2. A fusion protein possesses higher antitumor activity than combined therapy of antibodies with cytokine, or cytokine in monotherapy (IL-12). Physical combination of two biologically active proteins is likely to provide "correct" spatial positioning of cytokine in extracellular matrix of tumor microenvironment [70]. The represented data indicate the possibility to reduce an effective dose of cytokine and injection frequency compared to cytokine monotherapy.

Currently, there being studied simplified versions of fusion proteins based on incomplete antibodies, which can ease the product technology. However, they have a number of disadvantages:

lower antigen binding ability;

quicker washout period;

incomplete set of antibody functions (e.g., Fc-region provides induction of antibody-dependent cell-mediated cytotoxic activity).

Over the past decade MAb–C with various functional orientations have been produced and are under clinical trial. One of the directions in this area is the production of antibodies fused with two different cytokines – di-cytokine fusion proteins (Table 2). The source of the idea is the study on the combination of several cytokines, which indicate significant increase of antitumor activity [92–96].

Gene engineering enabled to produce recombinant antibodies using different expression systems — from bacteria to mammalian cells. The production of fusion proteins from plants with temporary expression is being developed intensively. There being searched combined molecules aiming to enhance therapeutic effect. Just a limited number of MAb–C succeeds in passing clinical trials, though their appearance at pharmaceutical market is just a matter of time.

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