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REVIEW

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EXPRESSION OF CD40 AND CD40L ON TUMOR CELLS: THE ROLE OF THEIR INTERACTION AND NEW APPROACH TO IMMUNOTHERAPY

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In the review the modern insights on the role of expression of CD40 and CD40L and the role of their interaction on tumor cells growth are analyzed. Information about the structure and biologic properties of these molecules and their interaction is presented. The question on the role of CD40/CD40L interaction is highlighted in two aspects – the possibility of tumor growth inhibition and its stimulation. According to the mentioned aspects, immunologic mechanisms providing tumor growth inhibition (the role of dendritic cells, macrophages, monocytes, cytotoxic T-lymphocytes, natural killer cells etc.), and also the possibility of apoptotic events are discussed. Possibility of tumor growth stimulation upon the influence of CD40/CD40L interaction that could occur in some cases is analyzed as well. The data of literature about new approaches to immunotherapy of cancer based on CD40/CD40L interactionare summarized.

***Key Words*: CD40, CD40L, CD40/CD40L interaction, tumor, immunotherapy.**

CD40 — a type I glycosilated phosphoglycoprotein with the molecular weight of 48 kDa — belongs to the superfamily of type I TNF receptors. This molecule was firstly identified on normal and transformed B-lymphocytes, and also on the cells of bladder tumor as early as at 80th of last century [1, 2].

The discovery of CD40 molecules on normal B-lymphocytes has encouraged the researches aimed on the estimation of their role in immunologic response that began after identification of the ligand for this molecule on T-lymphocytes — CD40L (CD154). As a result, it has been shown that CD40 and CD40L play a key role in intercellular interactions of the abovementioned two main populations of cells of immune system [3]. Later it has been revealed that CD40 is expressed also by a number of antigen-presenting cells (dendritic cells, monocytes, macrophages) eosinophils, basophils and also by keratinocytes, epithelial, neural and other types of cells, and its expression possesses pronounced costimulatory properties [4–6]. The structure of CD40 includes extracellular, transmembrane and intracellular parts. The patterns of CD40 structure allow to suppose that the molecule can act as a trimeric receptor able to activate different messengers, and its functional activity is manifested at the highest degree when CD40 acts as a trimeric receptor complex. The structure of CD40 molecule is presented on the Figure [5].

This information finally allows to determine CD40 as a structure playing an important role in different forms of immunologic response, because it is taking part in activation, proliferation, differentiation of different types of cells of immune system [5, 7–9].

EXPRESSION OF CD40 AND CD40L BY DIFFERENT TUMOR CELLS

During the study of CD40 and CD40L it became evident that these molecules may be expressed by a number of tumor cells, some of which may express CD40, other — CD40L, and some cells — both molecules. Presently it is known that CD40 is expressed by the cells of different breast carcinoma, nasopharyngeal, ovarian and intestinal carcinoma, melanoma, glyoma, hepatocellular carcinoma, lymphoma etc. However, whilst the role of CD40/CD40L interaction on the cells of immune system has been studied in detail, the role of such interactions on tumor cells is presently under active study and has not been clarified yet due to ambiguousness of the obtained data. For example, it has been demonstrated that CD40/CD40L interaction on many tumor cells (melanoma, different carcinoma, multiple myeloma, lymphoma etc.) may lead to inhibition of tumor cell growth, but in some cases may promote it, too [10–13].

The problem is complicated also by the fact that peculiarities of CD40 expression may be manifested in different ways toward different lines of the tumor. The study of CD40 expression by the cells of different melanoma lines, primary melanoma and metastases as well in different skin lesions that precede the development of melanoma, allowed to state a number of interesting facts: 1) CD40 expression did not possess regular character and in a number of cases it was not detected on the cells of some lines and freshly isolated

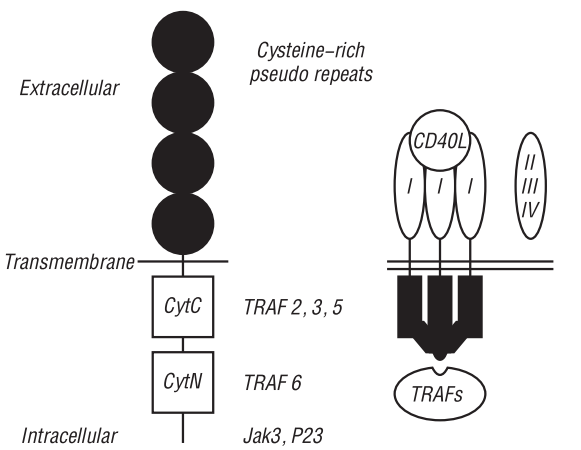

Figure. Structure of CD40 type I isoform (*a*) and functional CD40 receptor (*b*) [5]

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Abbreviations used: CTL – cytotoxic T-lymphocytes; GM-CSF – granulocyte-macrophage colony stimulating factor; IFNγ – interferon gamma; IL-2 – interleukine-2; LAK – lymphokine-activated killer cells; TNFα – tumor necrosis factor-α.

melanoma cells; 2) during progression of cancer, CD40 expression has been decreasing; 3) in a large number of cases CD40 was expressed by the cells of immunogenic melanoma, but practically never — by the cells of metastases; 4) CD40 expression increased after stimulation by IFNγ and TNF, but not IL-1β or CD40L [10]. There are interesting data showing that melanoma cells expressing CD40 were able to manifest themselves as co-stimulatory TCR of T-lymphocytes even in the absence of expression of co-stimulatory CD80 and CD86 molecules in mentioned cells. These data allowed to consider CD40 as one of the molecules, involved in cell-mediated stimulation of $CD4^+$T-lymphocytes activated by anti-CD3-antibodies. The authors of this work have demonstrated also that along with stimulation of $CD4^+$T-lymphocytes, CD40 expression by melanoma cells may promote their proliferation [4].

In another research, an interesting interpretation of the data on CD40 expression by the cells of 18 melanoma lines obtained from the patients with metastasis and primary tumors has been presented. In particular, the authors observed a significant percent of CD40 expressing cells in the majority of lines, only few of which demonstrated elevated CD40 expression upon the influence of IFNγ, but were insensitive for the action of GM-CSF, IL-2 or TNFα. The influence of anti-CD40-antibodies led to increased cell division, but did not elevate B.7 expression; these data allow to conclude on the important role of CD40 in biology of melanoma cells, but did not answer the question on the role of interaction between CD40 and its ligand in antitumor immunity [14].

There are interesting data obtained on the large material and dealing with the comparative study of CD40 expression by the cells of nevus and melanoma. It has been shown that in dermoepithelial zones of nevus, CD40 molecules are detected only in single cells whilst in dermal part they are not detected at all. At the same time in melanoma in a large majority of cases CD40 has been detected with the intensity dependent on the phase of growth; as a rule, CD40L has been found only in the cells expressing CD40 gene [15]. Comparative evaluation of the patterns of clinical course of melanoma didn't reveal the difference between CD40-positive and CD40-negative melanocytes, but there were found the differences in prognostic significance — the patients with CD40-negative tumors were characterized by shorter survival period. These results allow to consider CD40 as a prognostic marker for primary skin melanoma, and simultaneous expression of CD40 and CD40L — as a growth-promoting factor.

As it has been shown, CD40 is expressed by normal neural cells, and in CD40-deficient mice the dysfunction of neurons accompanied by a number of changes, in particular, morphological ones, elevated rates of DNA fragmentation and brain abnormalities, is observed — the facts allowing conclude that CD40 molecules play the role in the development of neurons and their protection *in vitro* and *in vivo* [16]. Such physiologic role of CD40 expression by neurons one should take into account upon evaluation of this expression in malignantly transformed cells.

The research of CD40 expression by neuroblastoma cells in parallel with such co-stimulatory molecules as CD80, CD86, B7H2, OX40L, 4-1BBL, has shown that on the primary neuroblastoma only some part of the mentioned co-stimulatory molecules is expressed, but CD40 was constantly expressed by the cells of human primary neuroblastoma and the cells of different lines. Transfection of neuroblastoma cells with IFNγ gene leads to expression of CD40 and induction of apoptosis upon incubation of cells with recombinant CD40L; the authors explained the observed death of tumor cells by activation of caspase-8 [17].

There are the data showing that CD40/CD40L interaction results in direct inhibition of the growth of human glioma cells *in vitro*. Incubation of glioma cells with recombinant sCD40L resulted in CD40 expression on freshly isolated cells as well as on the cells of one line from three studied, decreased tumor cell viability and increased apoptosis rate; these data made the grounds to discuss the possibility of the use of sCD40L for the treatment of CD40-positive glioma [18].

Different breast carcinoma cells may express CD40 and CD40L. The expression of CD40L on breast carcinoma cells has been revealed recently by Tong et al. [19]. Due to the scarce data, presently there is no sole theory on the role of expression of this molecule [19]. The study of expression of MHC class II antigens, CD40, CD80 and CD86 revealed different levels of expression of mentioned molecules on different breast carcinoma cell lines [20]. The authors find it difficult to evaluate the biologic meaning of expression of mentioned molecules, and suppose that it may be one of the reasons of tumor's escape from immunologic control (as it was mentioned above, analogous point of view has been proposed for melanoma cells, too).

Expression of CD40 by breast carcinoma cells and CD40/CD40L interactions have been studied by other authors, too. It has been noted that incubation of breast carcinoma cells with the soluble form of recombinant CD40L *in vitro* led not only to inhibition of cell proliferation, but also promoted inhibiting action of IFNγ. These results allowed to conclude on the possibility of clinical application of sCD40L for the therapy of breast cancer [12].

Upon evaluation of the role of CD40/CD40L interactions on the cells of breast cancer cell line it has been revealed that transfection of these cells with co-stimulatory molecule B.7 resulted in the activation of the mechanisms of antitumor defense. Despite the fact that co-transfection with CD40 and CD40L was not performed, the authors supposed that the defense mechanism in this case may be related to CD40/CD40L interaction resulting in carcinoma-specific activation of TCR [21].

To detect CD40 and CD40L expression on tumor cells, retrospective analysis of the results of immunohistochemical study of biopsy materials has been

performed. In particular it has been shown that in cancer of milk ducts and lobular form in the majority of cases CD40 expression was registered. CD40L was expressed at various levels more often in infiltrating forms of cancer than in carcinoma *in situ*. Tumors were infiltrated by lymphocytes, the majority of which were expressing CD40, whilst the minority — CD40L. That's why the authors concluded that lymphocytes infiltrating the primary tumor could possess a limited capacity to modulate directly tumor growth by CD40/CD40L interaction [19].

Expression of CD40 has been found also on the cells of other epithelial tumors. The study of ovarian cancer cells (cell lines as well as freshly isolated tumor cells) has shown that the cells of all lines are expressing CD40, and CD40 mRNA was revealed in each primary sample thus pointing that it may occur *in vivo* as well [22]. The high rate of the presence of CD40 in ovarian cancer cells allows to conclude that this molecule may be used as a target for the therapy of ovarian cancer.

Expression of CD40 at different levels and its dependence on cytokine action has been observed in the cells of human hepatocellular carcinoma and squamous cell carcinoma of head and neck. In the first case the level of CD40 expression was extremely low, so the authors did not find it reasonable to evaluate its influence on hepatocellular carcinoma cells. Moreover, according to the obtained data, CD40/CD40L-interaction did not affect the viability of hepatoma cells and the expression of co-stimulatory molecules CD54, CD80, CD86 and CD25 [23].

The study of CD40 expression the cells of human squamous cell carcinoma of head and neck has shown that the cells of all lines expressed CD40, EGF receptor, MHC class I antigens, CD95, but constant expression of such important regulatory molecules as CD80, CD86, CD25 wasn't observed. Analyzing the obtained results, the authors have concluded that, firstly, CD40 is a molecule regulating the growth of the cells of squamous cell carcinoma, and secondly, that CD40/CD40L interaction plays an important role in regulation of immunocompetent cells of lymph nodes, and high level of CD40L expression allows hypothesize that such ligand-receptor interactions are important mediators of the growth of squamous cell carcinoma cells [24].

The abovementioned data are evidencing on ability of tumor cells of different histogenesis (mainly mesenchymal and epithelial) and localization to express CD40 and CD40L. The reported data are showing also that in the majority of cases CD40/CD40L interactions on tumor cells leads to inhibition of tumor growth, but in some cases CD40/CD40L interaction may have stimulating effect as well. That's why it is difficult to evaluate biological role of CD40 and CD40L expression by tumor cells. Naturally, a number of questions appears, at the first hand, the next ones: 1) Why some tumors express CD40, the other ones — CD40L, and the third ones — both molecules? 2) Why the cells originated from the same tumor vary in CD40 expression level? 3) What are the conditions promoting an interaction of CD40-positive tumor cells with the cells of immune system? 4) Why CD40/CD40L interaction may influence tumor growth in different ways? To answer these questions, one should take into account that CD40 may affect tumor cell proliferation [25], and such influence may manifest itself in dependence on the properties of tumor cells and microenvironment. These questions as well as other ones should be studied further, and presently there are numerous data allowing to explain some mechanisms of the influence of CD40/CD40L interaction on tumor growth.

INHIBITION OF TUMOR GROWTH UPON INFLUENCE OF CD40/CD40L INTERACTION

The decisive fact of tumor growth inhibition due to CD40/CD40L interaction on tumor cells expressing the mentioned molecules has open a question on the mechanisms of such inhibiting influence. Analysis of the literature data on this topic allows to conclude that there are two main mechanisms of such influence on tumor growth. The first one is the induction of different immunologic processes participating in antitumor defence, and the second one is an induction of apoptosis. The study of mentioned mechanisms has shown that each of them may result from the activation of numerous processes, i.e. be multifactorial.

IMMUNOLOGIC MECHANISMS

As it is known, the central place in the induction of specific antitumor immunity is occupied by the activation of antigen-presenting cells, in particular dendritic cells, monocytes, B-lymphocytes which, as a rule, express CD40. Exactly these cells are providing the first and the most important stage of immunologic response — recognition of tumor antigens.

In the study of processes of recognition a lot of attention is devoted to professional antigen-presenting dendritic cells. There is a sufficient amount of data on the favourable influence of CD40/CD40L interactions on the functions of dendritic cells, the disturbance of which, as it is known, happens often upon the influence of different suppressing substances produced by tumor cells [26]. Using breast cancer cells, it has been demonstrated that CD40-ligation protects circulating dendritic cells from apoptosis caused by the action of such substances, by induction of expression of antiapoptotic molecule Bcl-2 [7]. Moreover, at these conditions not only the protection of dendritic cells from apoptosis is taking place, but the functions of populations of immature dendritic cells are promoted. It is of significance also that the activation of dendritic cells is accompanied by the elevation of IL-12 and INFγ production and enhanced cytotoxicity of killer cells. Intracellular mechanism of CD40 activation is accompanied by the activation of a number of transcriptional factors of protein origin, and this process occurs with the involvement of Toll-like-receptors [27].

Activation of dendritic cells is possible not only due to the interaction of recombinant CD40L or anti-CD40-antibodies, but also via interaction with tumor cells expressing CD40L. It has been proved in experiments on ovarian carcinoma cells transfected with CD40L gene with the use of adenoviral vector. Upon interaction of these cells with dendritic cells of peripheral blood, IL-12 production by dendritic cells was elevated [9].

For induction of immunologic response it is important to account that activation of CD40, expressed by **macrophages and monocytes**, is accompanied by their stimulation and activation of numerous immunologic mechanisms. For example, intraperitoneal administration of anti-CD40-antibodies activates macrophages that acquire ability to suppress proliferation of melanoma B-16 cells; after such activation macrophages begin to produce INFγ [28]. Further studies of these authors have shown that the activation of CD40 leads to increased expression of intracellular Toll-like-receptor (TLR9) in macrophages — the fact evidencing on involvement of this receptor in the process of activation of macrophages at mentioned conditions [29].

Stimulation of CD40 by anti-CD40-antibodies or soluble form of CD40L (sCD40L) enhances also the activity of monocytes, in particular in cervical carcinoma cells. This process is based on activation of NFkappa-B and MARK-dependent pathways, that leads to elevation of expression of surface molecules and activation of a number of intracellular processes related to antigen presentation and processing [30].

CD40/CD40L interaction plays an important role on **B-lymphocytes**, too; it is known that such interaction promotes their proliferation, differentiation, increases the level of expression of co-stimulatory molecules and elevates antigen presentation [31]. Interaction of CD40L with B-lymphocytes loaded with tumor peptides is capable to induce antitumor immunity. This statement has been proved by experiments using intravenous administration of CD40L to mice, whose B-lymphocytes were loaded by peptides — tumor antigens presented by HLA class I antigens [32]. At such conditions tumor growth retardation has been observed. As a result of activation of B-lymphocytes upon the influence of CD40L, an induction of specific T-cell response occurs, that (by opinion of the authors) develops due to the direct influence on B-lymphocytes, and cross one — on resident antigen-presenting cells.

Stimulation of CD40 on tumor cells is not limited by the activation of antigen-presenting cells and includes also other cells of immune system that participate at the further stages of the development of immunologic response. Presently it is known that activation of CD40 leads to stimulation of **cytotoxic T-lymphocytes (CTL), memory T-lymphocytes, natural killer cells (NK).**

After transfection of CD40L gene in multiple myeloma cells active generation of specific CTL and the tendency to increased Th1-cell response is observed [33]. There are interesting results of the studies carried out on cell lines of different carcinoma (bladder, pancreatic, breast carcinoma and melanoma) expressing CD40. The treatment of the cells with recombinant CD40L or anti-CD40-antibodies was accompanied by expression of ICAM, Fas-antigen on tumor cells, and stimulated production of IL-6, IL-8, GROα, GM-CSF and TNFα, whilst incubation of CD40-positive tumor cells with CD40L or anti-CD40-antibodies leads to significant inhibition of tumor cell proliferation, disturbance of cell cycle and decreased viability of the cells. On the base of obtained data, the authors have concluded that the presence of CD40 on carcinoma cells may be considered as an important factor of induction of tumor-specific T-cell response [34].

Activation of T-cell specific antitumor response is taking place also upon transfection of dendritic cells with CD40L; in these conditions $CD4^+$- and $CD8^+$T-lymphocytes are activated [35].

Ligation of CD40 induces antibody-dependent cytotoxicity of NK $CD56^+CD3^-$cells. Using multiple myeloma cells expressing CD40, it has been shown that action of humanizated anti-CD40-antibodies (SGN-40) on these cells mediates antibody-dependent cytotoxicity against myeloma cells via suppression of IL-6-dependent proliferation and apoptosis [36].

From the mentioned data one may conclude that CD40/CD40L interaction on tumor cells manifests itself by activation of different cells of immune system involved in antitumor defence.

APOPTOTIC MECHANISMS

The study of biologic role of CD40/CD40L interaction allowed to obtain the data evidencing on the fact that along with its positive influence on the functions of cells of immune system, it may take part in induction of apoptosis. Such results have been obtained in the studies of tumor cells of different etiology and histogenesis [5, 37, 38]. Then the question is rised on the mechanisms of CD40/CD40L-induced apoptosis. The important studies carried in parallel on normal and transformed cells have shown that upon activation of CD40, apoptosis is observed, as a rule, in malignant cells but not in normal ones [5, 39, 40]. Then the next question appeared — how these differences could be explained? Unfortunately, presently this question remains unanswered, but some facts allow to explain the phenomenon in part.

As it was revealed, different apoptotic stimuli influence different genes in normal and transformed cells. For example, the study of normal keratinocytes and oral cancer cells of Tu183 line has shown that upon action of different proapoptotic stimuli, expression of different proapoptotic genes occurs as well as the gene coding for CD40 [40].

The study of normal and malignantly transformed epithelial cells of urinary tract has demonstrated that upon interaction of malignant cells with mCD40L, but not sCD40L quick elevation and stabilization of TNF-associated factor 3 (TRAF-3) and activation of

caspases-9 and -3 is taking place. Contrary to this, in normal cells CD40L is not inducing apoptosis but leads to quick decrease of activity of TRAF-3 and TRAF-2 [39]. These data are of interest because, firstly, they demonstrate the differences in molecular mechanisms of influence of mCD40L on normal and transformed cells, and secondly, they reveal a new way for mCD40L-induced apoptosis. According to recently published data, another factor associated with TNF-receptor family may decrease proliferation and promote apoptosis of myeloma cells, — TRAF-6 [41]. The authors consider this factor as a new molecular target for blocking signal transduction necessary for survival and proliferation of the cells of multiple myeloma.

The functional meaning of CD40 expression on normal and transformed cells of urinary tract has been studied also by other authors. In experiments the cells were incubated with CD40L, TNFα and anti-Fas-antibodies; it has been shown that CD40L leads to apoptosis of transformed cells, but not of normal cells where induction of apoptosis requires the presence of other TNFR-agonists. It has been noted that the decrease of expression level of CD40 could be considered as an important mechanism of the development and progression of carcinoma [42].

The evidence on possibility to induce apoptosis at the conditions of CD40 activation was demonstrated by many authors who have shown that the different mechanisms could be involved in this process. In early publications it was shown that CD40/CD40L-induced apoptosis may occur with the involvement of caspase-dependent and caspase-independent mechanisms. In particular, the development of apoptosis upon activation of CD40 on multiple myeloma cells was not associated with DNA fragmentation and enhanced CD95 expression [37].

Ability of CD40/CD40L interaction for induction and modeling of CD95-dependent apoptosis has been demonstrated on neuroblastoma that is known to possess the decreased level of expression of antigens HLA classes I and II; upon the influence of recombinant CD40L apoptosis is induced via caspase-8-dependent mechanism [43].

The study of squamous cell carcinoma of head and neck has demonstrated that CD40-ligation decreased the rate of cell proliferation by induction of spontaneous and Fas-induced apoptosis and also EGFr-dependent inhibition of proliferation [44].

There are also the data evidencing that CD40-ligation is not related to development of CD95-mediated apoptosis. In the study of different ovarian cancer cells (conserved and freshly isolated ones, from primary and secondary tumors) it has been stated that there are the differences in the levels of CD40 expression between ascitic cells and cells from solid forms as well as between primary and secondary tumors [38].

It's interesting to note that despite the fact that CD40 belongs to the family of TNF-receptors, CD40/CD40L interaction is accompanied only with activation of PI3k/Akt-signal cascade, but not NFkappa-B activation contrary to other members of the family [45].

CD40/CD40L interaction may play a role not only in solid tumors but lymphoproliferative diseases as well, in particular B-cell chronic lymphocytic leukemia a number of which express IL-21 receptor. Expression of this receptor is associated with the expression of CD40, and upon the influence of IL-21 in the cells of B-cell chronic lymphocytic leukemia, caspases 8 and 3 are activated, proliferation is suppressed and apoptosis is induced [46]. The authors consider these facts as a new mechanism of regulation of the balance between cell survival and death of B-lymphocytes at chronic lymphoid leukemia.

Summarizing the mentioned above data on the mechanism of inhibiting influence of CD40/CD40L interaction on tumor growth, one may conclude that these data are convincing. However, one should take into account the point of view of some authors who suppose that the inhibition of tumor growth resulting from CD40/CD40L interaction in many cases may be caused by combined effect including an induction of apoptosis and stimulation of immunologic processes starting from recognition of tumor antigens [5, 30].

STIMULATION OF TUMOR GROWTH UPON CD40/CD40L INTERACTION

As it was mentioned above, in many cases CD40/CD40L interaction leads to inhibition of tumor growth. Along with this, there are also some data that evidence on CD40/CD40L interaction that may result in tumor growth stimulation. The study of the phenomenon of such stimulation is important because firstly it is necessary to determine the methodology of the therapy based on CD40 activation. The data on this topic are scarse, and indicate that different mechanisms may be involved in tumor growth stimulation upon CD40 activation.

One of such mechanisms is the secretion of cytokines promoting tumor growth. In the study of multiple myeloma cells, the elevation of secretion of VEGF — active factor of angiogenesis — has been observed in parallel with increased migration of myeloma cells, thus indicating the role of CD40 in regulation of their homing and angiogenesis. Comparative study of different myeloma cell lines expressing wide type p53 and mutant ones has shown that VEGF expression was significantly higher in the cells expressing mutant p53 gene pointing on participation of the gene in VEGF secretion [36, 47].

There are the data demonstrating that stimulation of tumor growth upon CD40 activation may be caused by production of IL-6. These data were obtained in the research of myltiple myeloma cells expressing CD40, and plasmatic cells of leukemia and stromal cells. Transfection of myeloma cells with CD40L gene leads to the increased IL-6 secretion by these cells and stimulation of cell growth in autocrine or paracrine fashion [48]. Possible participation of cytokines in tumor growth stimulation has been noted by many authors [49].

Tumor growth stimulation upon CD40 activation may occur also via elevation of heterotypic and homotypic adhesion. In the research of myltiple myeloma cells such stimulation was associated with the expression of a number of surface structures and translocation of 86 kDa subunit of Ku-antigens involved in cell adhesion [48, 50].

There are important data on the ability of CD40-activation to stimulate multiple drug resistance that were obtained in the study of non-Hodgkin's lymphoma cells and breast cancer cells. According to the data, CD40L induced multiple drug resistance (to doxorubicine, etoposide, vinblastin etc.) in mentioned cells by different ways: in non-Hodgkin's lymphoma cells — by caspase-dependent and -independent ways, while in breast cancer cells — only by caspase-dependent one. In the next studies on non-Hodgkin's lymphoma cells and carcinoma cells co-cultivated with irradiated CD40-positive L-cells it has been shown that in carcinoma cells caspase-independent apoptosis is realized, while in non-Hodgkin's lymphoma cells the activity of caspases-5-7 was elevated manifold compared to the initial level. The negative influence of CD40 activation has been proved by the fact that the treatment with anti-CD40-antibodies prevents the development of drug resistance [51, 52].

Using gastric carcinoma cells it has been shown that activation of CD40 with soluble form of CD40L significantly inhibits Fas-dependent and drug-induced apoptosis and also increases the motility of CD40-positive tumor cells, that finally leads to elevated survival of gastric carcinoma cells [53].

It has been demonstrated that the stimulation of bladder carcinoma cells expressing CD40 with CD40L protects the cells from apoptosis and increases the survival rate, and the mechanism of cell growth stimulation is related to the decrease of CD95-dependent apoptosis [54].

Incubation of lung cancer cells of A549 line expressing CD40 with sCD40L results in high level expression of CD54, TNFR1 and CD95L. The authors have noticed that such effect is dependent in large part on the phase of cell cycle, and concluded that sCD40L may inhibit proliferation, alter phenotype of A549 cells and disturb the expression of apoptosis-associated genes, in particular these controlling expression of Bax [55].

Stimulation of cell growth, elevated cell survival and neovascularization have been registered also on sarcoma Kaposi cells treated with anti-CD40-antibodies; moreover, the induction of tumor cell migration and inhibition of vincristin-induced apoptosis were shown, too [56].

Activation of CD40 may lead to inhibition of apoptosis with the involvement of another caspase-dependent mechanism mediated by PPAR receptor and activation of NFkappa-B, as it has been shown on murine B-lymphoma cells of WEHI-231 line [57].

Concluding the abovementioned data, one could state that activation of CD40 may lead to tumor growth stimulation by different ways. This fact is showing that CD40 expression like that of other surface structures of tumor cells (cytokine receptors etc.) may manifest itself in dependence on the properties of the tumor and tumor microenvironment.

NEW APPROACHES TO IMMUNOTHERAPY BASED ON CD40/CD40L INTERACTION

Despite incomplete knowledge on biologic role of CD40 expression on tumor cells and influence of its activation on tumor growth, the large part of data are evidencing on its potential role as a target for cancer immunotherapy. The data about negative influence of CD-40 ligation may be used as well for the development of means for its blocking.

As it was mentioned above, CD40/CD40L interaction leads to induction of different mechanisms of antitumor defence; the development of means for immunotherapy is based exactly on these mechanisms. Presently there are the next ways based on the activation of CD40/CD40L interaction: 1) stimulation of antigen-presenting cells; 2) induction of CD40/CD40L interaction on the surface of tumor cells and their gene-engineering modification; 3) preparation of the vaccines on the base of CD40/CD40L interaction; 4) combination of CD40 activation with other therapeutical approaches.

Activation of CD40 on antigen-presenting cells

From the above mentioned reports one may conclude that activation of immune system cells, mainly antigen-presenting ones, has been performed in different ways: influence of CD40L or anti-CD40-antibodies and gene-engineering modification of dendritic cells with the use of different viral vectors. In a large number of studies, the authors have observed tumor growth inhibition and have concluded that the reported facts are providing rationale for clinical application of such approach. In particular, such data were obtained in the studies of different tumors — neuroblastoma, breast cancer, cancer of head and neck, melanoma [7, 29, 44, 58, 59].

For activation of antigen-presenting cells humanizated anti-CD40-antibodies (SGN-40) are used. For example, the treatment of human multiple myeloma cells of different lines has been shown to significant increase of apoptosis rate and decreased expression of IL-6 receptor. The authors supposed that the results are making grounds for clinical application of SGN-40 [36].

Transfection of immature dendritic cells with CD40L using adenoviral vector promoted their maturation and elevated the cytotoxicity of $CD4^+$T-lymphocytes with killer activity. Such vaccine introduced intratumorally or in distant sites sharply suppressed the growth of myeloma [33].

Induction of CD40/CD40L interaction on the surface of tumor cells and their gene-engineering modification

The efficacy of such approach for immunotherapy has been proved by the studies carried on different mod-

els: small cell lung cancer, cervical, gastric, and bladder cancer, prostate adenocarcinoma [30, 54, 60].

After transfection of rat adenocarcinoma cells of TRAMP-CD line with CD40L gene using adenoviral vector, the derease of tumor cell viability and induction of apoptosis has been observed *in vitro* and *in vivo*, allowing to conclude on the perspectiveness of such therapy approach [61].

Introduction of CD40L gene in tumor cells (mastocytoma P815) potentiated the activity of dendritic cells, accelerated their maturation, induced antitumor defence pointing on the possibility to use this approach for anticancer gene therapy [62].

Similarly, introduction of blastoma cells transfected with CD40L gene using retroviral vector resulted in decreased tumor volume *in vivo*, increase of the number of $CD4^+$- and $CD8^+$T-lymphocytes as well as elevated survival of animals [63]. The authors have noted that after administration even of low amount of these modified tumor cells, prolonged systemic antitumor immunity has been preserved.

Preparation of vaccines on the base of CD40/CD40L interaction

As it is known, the use of dendritic cells loaded with tumor peptides, tumor cell lyzates, RNA, apoptotic tumor cells, or gene-engineering modification of dendritic cells is presently considered as the most perspective immunotherapeutical approach [64]. This approach consideres the use of dendritic cells loaded with tumor antigens upon activation of CD40, and some experience of preparation and application of such vaccines has been accumulated.

The use of dendritic cells or monocytes loaded with tumor peptides (MART-1/MelanA) and transfected with CD40L was accompanied by expression of costimulatory molecules, elevation of immunogenic properties of tumor peptides and generation of cytotoxic $CD8^+$T-lymphocytes [35].

Using mastocytoma P815 model it has been shown that immunization of animals with bone marrow activated dendrititc cells loaded with tumor peptides of mastocytoma (P198 and PIA) promoted interaction with CD40L cytotoxic T-lymphocytes (fibroblasts transfected with CD40L gene were used as a source of CD40L-signal). Taking into account the pronounced antitumor response, the authors have concluded that vaccination with dendritic cells could be applied on clinical practice [65].

If dendritic cells transfected with melanoma antigen MART-1 using adenoviral vector were administered to melanoma-bearing mice, an accelerated maturation of dendritic cells and an induction of CTL-dependent antitumor defence has been observed [66].

CD40/CD40L-interaction may play an important role in realization of effects of vaccines from tumor peptides. In these cases the therapeutical effect is provided by the involvement of cytotoxic T-lymphocytes, thus making grounds for use of CD40-stimulating agents as components of antitumor vaccines [67].

Another promising approach is the use of antitumor vaccine with autologic tumor cells transfected with recombinant CD40L [68], and it is supposed that the use of such vaccine is excluding the risk of the development of autoimmune pathology and could be proposed for clinical application.

For vaccine preparation one could use also CD40L-activated B-lymphocytes loaded with tumor peptides that are presented by antigens MHC class I, and stimulating different immunologic mechanisms [Ritchie].

At last, the approach based on the use of B-lymphocytes from peripheral blood of healthy donors, stimulated by CD40/CD40L-interaction and loaded with tumor antigens should be mentioned. These B-cells possess strongly elevated antigen-presenting patterns. Such vaccine was obtained with the use of melanoma antigens or melanoma cell lyzates, and pronounced promotion of presentation of the antigens with involvement of MHC class II antigens to $CD4^+$T-lymphocytes was observed [69].

Combination of CD40 activation with other therapeutical approaches

In recent years there is a number of reports on high efficacy of combination of activation of CD40/CD40L interaction with other therapies. The most promising is its combination with chemotherapy. For example, activation of CD40 in a complex with nucleoside analog hemcytavine has been used for treatment of different solid tumors. In these conditions it resulted in an expansion of $CD8^+$T-lymphocytes, whilst chemotherapy promoted cellular antitumor immunity [3].

Preincubation of murine breast cancer cells transfected with CD40L using adenoviral vector, with carboplatine significantly elevated antitumor effect compared to that for modified tumor cells only [9].

CD40/CD40L interaction was shown to occupy an important place also in the case of vaccination with irradiated melanoma cells and adjuvants [70].

A number of studies proved an efficacy of CD40-activation in the combination with other immunotherapy approaches (immunomodulators, adoptive LAK-therapy). For example, the combined use of humanizated anti-CD40-antibodies along with immunomodulator lenalidomide (pretreatment of myeloma cells with this immunomodulator) promoted the lyzis of autologous cells of multiple myeloma and restored the sensitivity of resistant myeloma cells [36].

Promotion of antitumor effect upon CD40-activation has been observed also in the case of administration of oligonucleotide immunomodulator CpG, that was accompanied by increased production of INFγ, IL-12, NO by macrophages in parallel with elevation of their cytotoxic action leading to inhibition of growth of melanoma B16; activation occurred upon interaction of CD40-positive tumor cells and TLR9 of macrophages. The efficacy of such therapy has been documented *in vivo* even upon the development of immunologic insufficiency [28, 29].

There is an interesting study on a new strategy for combined use of dendritic cells and LAK (activation

with IL-2 or anti-CD3-antibodies), based on co-cultivation of dendritic cells and LAK isolated from peripheral blood of the patients with metastases and expressing CD40L; such approach significantly increased the activity of dendritic cells which antigen-presenting capacity was elevated two fold [71]. One should note that the effect has been achieved without gene modification and application of monoclonal antibodies.

Combined use of CD40 and cytokines may be considered as well. In a series of studies performed by Murphy et al. [72], the data on efficacy of the combined use of CD40 activation and IL-2 were reported. The combined use of IL-2 and CD40 activation was shown to be effective also in the influence on hemopoietic cells — it has been shown *in vivo* on chimeric mice carrying transplanted renal tumors [73]. In later studies it has been shown that the mentioned effect of the combined use of CD40 activation and IL-2 is related mainly to IFNγ-production. The authors have pointed to an important fact — the elevated IFNγ production is especially effective at early stages of formation of antitumor defence, but at later ones (at certain conditions) it may decrease the level of antitumor response [74].

For example, a pronounced antitumor response has been observed upon introduction of CD40 and IL-2 to mice with renal carcinoma and metastases whilst therapeutic efficacy of separate administration of CD40 or IL-2 was absent [72].

The methods of combined application of anti-CD40-antibodies and irradiation for combined treatment of B-lymphoma are developed [73].

Immunotherapy with the use of CD40 and CD40L in different variants is already used for treatment of some oncologic diseases in clinical practice. For example, in the cases of lymphoproliferative disorders (leukemia, lymphoma, multiple myeloma etc.) it has been shown that binding of CD40 with its natural ligand resulted in modulation of transformed B-lymphocytes [33]. On two lymphoma models it was demonstrated that combination of anti-CD40-antibodies with irradiation is accompanied by therapeutic effect mostly expressed. In the cases of highly differentiated lymphoma, where therapeutic efficacy depends on the doses of anti-CD40-antibodies, doses of irradiation and tumor volume, whilst antitumor effect is mainly related to cytotoxic T-lymphocytes [75].

Therapy with the use of CD40-stimulation has been applied on the treatment of child acute lymphoblastic leukemia [76]. The authors received new data on stimulation of the cells of bone marrow or peripheral blood by CD40L or IL-4 showing that after co-cultivation of leukemia cells with these stimulatory molecules, expression of different co-stimulatory molecules and of antigens MHC classes I and II could be detected. CD40L-activated leukemia cells acquired an ability to be transformed into dendritic ones and induced proliferation of allogeneic T-lymphocytes. These activated cells could be used for preparation of antitumor vaccine. It is important to note that after transplantation of these cells, the minimal residual effects were observed, and the patients with primary drug resistance acquired sensitivity to therapy [74]. The authors supposed that no necessity for gene modification of tumor cells is an advantage of such therapeutical approach. Other authors share this opinion [31, 77].

Discussing the clinical aspects of CD40 use, one should take into account the data on elevation of sCD40 level in some hematopoietic malignancies (acute myeloid leukemia, myelodysplastic syndromes, chronic lymphocytic leukemia, multiple myeloma) that is associated with poor prognosis, in particular, in multiple myeloma and acute myeloblastic leukemia; these facts could be important for therapy of malignant diseases of hematopoietic and lymphoid tissues [78].

Preclinical trials on therapy of disseminated solid tumors, non-Hodgkin's lymphoma with the use of recombinant CD40L introduced subcutaneously at the maximal tolerated dose have shown that such therapy resulted in prolonged remission [79].

Concluding the presented materials, one may state that the study of CD40/CD40L interaction upon cancer development is a comparatively new discipline that is actively developed and possesses important aspects: 1) widening the knowledge on interaction of the cells of immune system and tumor cells with the involvement of CD40 and CD40L; 2) characterization of bilogy of tumor cells accounting CD40 or CD40L expression; 3) development of new approaches to immunotherapy that already received some clinical significance.

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ЭКСПРЕССИЯ CD40 И CD40L НА ОПУХОЛЕВЫХ КЛЕТКАХ: РОЛЬ ИХ ВЗАИМОДЕЙСТВИЯ И НОВЫЕ ПОДХОДЫ К ИММУНОТЕРАПИИ

В обзоре представлены современные данные об экспрессии CD40 и CD40L опухолевыми клетками и значении взаимодействия указанных молекул на этих клетках. Приведена информация о структуре и биологических свойствах этих молекул и их взаимодействия. Вопрос о взаимодействии CD40/CD40L рассматривали в двух аспектах — возможность ингибиции роста опухоли и стимуляции. В соответствии с этим изучают иммунологические механизмы, обеспечивающие торможение роста опухоли (роль дендритных клеток, макрофагов, моноцитов, цитотоксических Т-лимфоцитов, естественных киллеров и др.), а также возможность развития апоптоза. Рассматривают возможность стимуляции роста опухоли под влиянием CD40/CD40L взаимодействия, что отмечают в отдельных случаях. Обобщены существующие данные доступной литературы о новых подходах к иммунотерапии на основе анализа механизмов вышеуказанного взаимодействия.

***Ключевые слова*: CD40, CD40L, CD40/CD40L-взаимодействие, опухоль, иммунотерапия.**