Cancer Immunol Immunother (2013) 62:949–954
DOI 10.1007/s00262-013-1427-5

FOCUSSED RESEARCH REVIEW

CD40 immunotherapy for pancreatic cancer

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Received: 10 September 2012 / Accepted: 11 February 2013 / Published online: 16 April 2013

Abstract Pancreatic ductal adenocarcinoma (PDA) is a highly aggressive and lethal cancer which is poorly responsive to standard therapies. Although the PDA tumor microenvironment is considered especially immunosuppressive, recent data mostly from genetically engineered and other mouse models of the disease suggest that novel immunotherapeutic approaches hold promise. Here, we describe both laboratory and clinical efforts to target the CD40 pathway for immunotherapy in PDA. Findings suggest that CD40 agonists can mediate both T-cell-dependent and T-cell-independent immune mechanisms of tumor regression in mice and patients. T-cell-independent mechanisms are associated with macrophage activation and the destruction of PDA tumor stroma, supporting the concept that immune modulation of the tumor microenvironment represents a useful approach in cancer immunotherapy.

This paper is a Focussed Research Review based on a presentation given at the 10th annual meeting of the association for cancer immunotherapy (CIMT), held in Mainz, Germany, May 23 to 25, 2012. It is part of a CII series of Focussed Research Reviews and meeting report.

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Keywords Tumor immunity · CD40 · Pancreatic cancer · Macrophages · T cells · CIMT 2012

CD40 immunobiology

The cell surface molecule CD40 is a member of the tumor necrosis factor receptor superfamily and is broadly expressed by immune cells, in particular B cells, dendritic cells (DC), and monocytes, as well as other normal cells and some malignant cells [1–3]. CD40 lacks intrinsic signal-transduction activity and mediates its effects via a series of downstream adapter molecules. As a critical regulator of cellular and humoral immunity, signaling via CD40 triggers activation or "licensing" of antigen-presenting cells (APC) both in vitro and in vivo and physiologically, represents a major component of T cell help [4]. CD40 ligand (CD154) is the primary ligand for CD40 and is expressed by activated T cells [3, 5]. CD40 ligation of DC upregulates surface expression of costimulatory and MHC molecules, triggers the release of proinflammatory cytokines, and enhances T-cell activation [3, 6].

More than 10 years ago, agonist CD40 antibodies were found to mimic the signal of CD40 ligand and were capable of substituting for the function of $CD4^+$ helper T cells in murine models of T-cell-mediated immunity [7–9]. In these experiments, CD40 agonists rescued the function of APC in tumor-bearing hosts and restored effective immune responses against tumor antigens. Moreover, agonist CD40 antibodies were found to overcome T-cell tolerance in tumor-bearing mice, and by enhancing the effects of vaccines, agonist CD40 antibodies have proven to be capable of facilitating the development of potent cytotoxic T-cell responses [10–12].

Importantly, data from multiple preclinical models demonstrate synergistic enhancement from combining

CD40 agonists with cytotoxics especially chemotherapy [11–17]. In these experiments, chemotherapy may be functioning as a "vaccine" by presumably releasing tumor antigens in an immunogenic fashion, potentially explaining in part the improved efficacy of CD40 agonists when given after, but not before, chemotherapy [13].

Translation to the clinic

There have been many efforts to translate CD40 immunotherapy to the clinic using agonistic CD40 monoclonal antibodies (mAb) [1, 18]. Three such mAb which have been the most extensively tested vary in engineering, isotype, and strength of agonistic signal [18]. CP-870,893 (Pfizer/VLST) is a fully human IgG2 and a strong agonist and has shown anti-tumor activity in patients with solid tumors including melanoma and pancreatic cancer. Dacetuzumab (Seattle Genetics) is a humanized IgG1 which is considered a weak agonist and has shown efficacy in a range of hematological malignancies, especially lymphoma, but there are no current clinical trials with this mAb. Chi Lob 7/4 is an intermediate CD40 agonist and chimeric IgG1 (University of Southampton); it is currently undergoing phase I evaluation in patients with solid tumors and lymphoma.

Our group has investigated CP-870,893 in detail in a series of clinical trials since 2007 [18].CP-870,893 activates human DC and B cells without the necessity of further crosslinking in vitro and has activity in human tumor xenograft experiments in vivo [19–21].Experience from more than 150 cancer patients treated with CP-870,893 indicates that intravenous administration is well tolerated at a maximum dose of 0.2 mg/kg [22–24]. The most commonly observed adverse event is a modest cytokine release syndrome which can be readily managed in the outpatient setting. Clinical responses have been observed both with CP-870,893 as a single agent and in combination with chemotherapy. In the first-in-human study of 29 advanced cancer patients treated with a single infusion of CP-870,893, four patients—all with metastatic melanoma—had a partial response as defined by RECIST criteria [22]. One of these patients, who went on to receive several repeated infusions of CP-870,893 about every other month for a year, continues to have an ongoing complete response more than seven years since enrollment (Robert Vonderheide and David Bajor, unpublished). More recently, 32 patients with advanced cancer, mostly metastatic melanoma, received CP-870,893 in combination with carboplatin and paclitaxel. In this study, performed in collaboration with Omid Hamid (Angeles Clinic) and Anthony Tolcher (START), the partial response rate was 20 % among evaluable patients, which includes tumor responses in patients with tumors that were otherwise chemotherapy refractory [25].

There are concerns from experimental preclinical work that agonist CD40 mAb may cause significant toxicity or promote tumor growth [26], but most of these concerns have not been realized in a clinically significant way. The most common side effect for CP-870,893 has been a moderate, transient cytokine release syndrome, managed in the outpatient setting [22, 23, 25]. Autoimmune reactions have not been observed, including no cases of colitis. Noninfectious inflammatory eye disorders have been observed with dacetuzumab [27]. Agonist CD40 mAb have also triggered mild elevations in liver enzymes but importantly in the absence of liver necrosis, hemolysis, or disseminated intravascular coagulation. As an alternative and to address these concerns of systemic administration and toxicity, local administration of CD40 mAb is one approach with interesting preclinical supporting data [28].

CD40 antibody therapy for pancreatic cancer

Recently, we tested the hypothesis that therapeutic CD40 activation might hold promise for patients with pancreatic ductal adenocarcinoma (PDA). PDA is a highly aggressive and lethal cancer that is refractory to most standard therapies. Over the last decade, only one new drug for patients with metastatic PDA has been approved in the United States, but this drug (erlotinib) extends survival on average by only a couple of weeks when combined with gemcitabine. In contrast, strategies to induce tumor immunity in patients with PDA have, in some cases, produced encouraging responses [29–31]. In collaboration with Pfizer Corporation, we therefore tested the combination of CP-870,893 and gemcitabine in patients with chemotherapy-naïve advanced pancreatic adenocarcinoma in a two-center trial at the University of Pennsylvania (PI, Peter O'Dwyer) and Indiana University (PI, Elena Chiorean) [24]. This trial combined standard-of-care gemcitabine (three weekly infusions with 1 week off per cycle) and CP-870,893 administered 2 days after the first dose of gemcitabine of each cycle. Although gemcitabine alone historically has produced a partial response of approximately 5 % or less in this setting [32], we observed 4 patients out of 21 with a partial response (with a fifth patient developing a partial response after receiving only a single dose of CP-870,893, as detailed elsewhere [24]). Eleven other patients had RECIST-defined stable disease as the best response on this study. A follow-up clinical study of CP-870,893 with gemcitabine is now underway for patients with resectable PDA at the University of Pennsylvania (NCT01456585).

Mechanistic studies in a murine model of pancreatic cancer

To understand the mechanism of action of combining agonist CD40 antibodies with gemcitabine chemotherapy in PDA, we studied a genetically engineered mouse model of pancreatic cancer that was developed at the University of Pennsylvania by David Tuveson and colleagues [33]. This model combines the targeted expression of a *Kras* mutation with a mutation in the tumor suppressor *p53*. These genetic lesions are targeted to the pancreas using a Cre–Lox approach, and immunologically, these animals ("KPC mice") offer an outstanding model system for the study of immunotherapeutic strategies for three reasons: First, mice develop tumors in the setting of a competent host immune system; secondly, tumor development is associated with a strikingly similar desmoplastic stromal reaction to that observed in human PDA; and thirdly, KPC mice develop not only invasive local lesions but also metastatic lesions, thereby reproducing the human condition with extremely high fidelity. Our group has previously shown that in such genetically engineered mice, suppressive cells of the host immune system appear early during pancreatic tumorigenesis, precede, and outweigh antitumor cellular immunity, and likely contribute to disease progression [34, 35]. Using an agonist rat anti-mouse CD40 mAb as a prototype for CP-870,893, we investigated the mechanism of CD40 therapy in the KPC model [24].

Our studies provide evidence of a role for both T-cell-dependent and T-cell-independent immune mechanisms induced by CD40 agonistic agents (Fig. 1). In these experiments, littermate mice were injected subcutaneously with a pancreas tumor cell line derived from the KPC model, and after tumors became palpable, tumor-bearing mice were treated with the agonist CD40 mAbFGK45 (vs. isotype control antibody) administered 48 h after the infusion of gemcitabine (vs. PBS), a regimen and schedule previously identified to capture and exploit the "vaccine effect" of CD40 agonists in combination with chemotherapy [13]. Whereas tumors grew progressively in control-treated animals, more than 80 % of mice receiving combination treatment with CD40 and gemcitabine treatment were found to undergo major tumor regression (Gregory Beatty and Robert Vonderheide, unpublished). No regressions were observed with gemcitabine alone, although a small fraction of mice treated with only CD40 mAb did show tumor regression. Importantly, this treatment effect was associated with a massive influx of T cells into regressing PDA tumors, which was not observed in control-treated tumors. When host mice were depleted of $CD8^+$ and $CD4^+$ T cells prior to treatment with CD40/gemcitabine, tumor growth was restored, demonstrating a role for T cells in the therapeutic response elicited by CD40 in combination with gemcitabine.

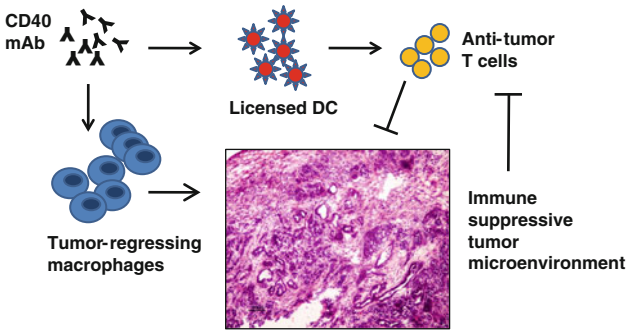

Fig. 1 CD40 agonists can mediate both T-cell-independent and T-cell-dependent immune mechanisms of tumor regression in pancreatic cancer. The former mechanism involves systemic activation of macrophages that infiltrate the tumor, become tumoricidal, and facilitate the depletion of tumor stroma. In combination with chemotherapy, CD40 agonists can also activate T-cell immunity and mediate major tumor regression; however, anti-tumor T-cell responses can be inhibited by suppressive elements in the tumor microenvironment. A scientific priority going forward is to understand whether blockade of these inhibitory micro-environmental mechanisms will enable adequate priming of an adaptive immune response in concert with CD40 activation in PDA

In a second series of experiments, we treated tumor-bearing KPC mice with the CD40 mAbFGK45 and gemcitabine (on the same schedule as in the implantable studies). Major tumor regressions were noted in 30 % of mice based on serial three-dimensional ultrasonography, similar to the objective response rate seen in patients [24]. Treatment with CD40 alone reproduced the same rate of tumor regression, whereas no tumor regression was observed with gemcitabine alone. In striking contrast to our implantable tumor studies, depletion of $CD4^+$ T cells, $CD8^+$ T cells, or both did not impact the regression rate observed with CD40 mAb in tumor-bearing KPC mice. These findings indicate that CD40 activation can also elicit a potent T-cell-independent mechanism of tumor regression. Given that PDA tumors are rich in infiltrating macrophages that express CD40, we hypothesized that tumor regression was dependent on macrophage activation. In support of this hypothesis, previous studies have suggested that CD40-activated macrophages can inhibit tumor growth, although IFN-gamma was essential, highly indicative of so-called M1 macrophages [36, 37]. While the administration of CD40 in tumor-bearing KPC mice did not produce a significant change in the magnitude of macrophages within the tumor microenvironment, a transient change in macrophage activation was seen within 24–48 h of treatment [24]. For example, the expression of both CD86 and MHC class II on tumor-associated macrophages was observed to increase significantly, but transiently, following CD40 mAb therapy in tumor-bearing KPC mice. We, therefore, depleted tumor-bearing KPC mice of systemic macrophages using clodronate-

encapsulated liposomes (CEL) and observed that CEL treatment prior to the administration of CD40 mAb abrogated CD40-mediated tumor regression in KPC mice.

In our studies to understand the mechanism of macrophage-dependent tumor regression induced by CD40 activation, we found that macrophages isolated from the pancreas of tumor-bearing KPC animals treated in vivo with CD40 mAb were capable of lysing tumor cells in vitro, correlating with in vivo observations of cleaved caspase 3 expression in focal areas of tumor at 18 h after treatment with CD40. Moreover, CD40 treatment promoted involution of the tumor stroma and degradation of tumor matrix at this early time point, an effect that was blocked by treatment with CEL. Importantly, these findings reinforce that CD40 immune therapy is not necessarily dependent on T cells and that the CD40 pathway can be harnessed to restore tumor immune surveillance by targeting tumor-infiltrating macrophages involved in cancer inflammation. Data recently reported from other experimental murine systems further support this notion [38].

Beyond the therapeutic implications of these findings, these results also suggest that a majority of macrophages in the PDA tumor microenvironment are actively "tumor promoting" and that in some respects, tumor regression observed following CD40 activation of macrophages reflects reversal of this tumor-promoting activity; however, because CD40 activation alone was sufficient for tumor regression, this may indicate that some amount of M1-type macrophages are present in PDA tumors. To begin to test the role of macrophages in PDA oncogenesis, we subcutaneously implanted a PDA tumor cell line derived from the KPC model in syngeneic mice. Host mice were either pretreated with CEL, to clear systemic macrophages, or given control liposomes. We found that PDA cells grew progressively in mice receiving control liposomes but failed to grow in CEL-treated mice (Robert Vonderheide and David Bajor, unpublished). This lack of tumor growth was associated with systemic depletion of macrophages, underscoring a tumor-promoting role for macrophages in PDA, as has been observed in other cancers [39–41].

The immune reaction associated with PDA is marked by tumor-infiltrating macrophages and immature myeloid cells that co-express the cell surface molecules CD11b and Gr-1. We recently reported a role for CD11b + Gr-1 + myeloid cells in regulating PDA tumor growth [42]. For both macrophages and immature myeloid cells, tumor-derived GM-CSF was found to drive infiltration into nascent PDA tumors, which could be abrogated with either GM-CSF-neutralizing antibody or knock down of GM-CSF expression in the tumors. Interestingly, tumor growth was rescued in the absence of $CD8^+$ T cells even without GM-CSF in the local tumor microenvironment. These results and those of others [43] suggest that an inflammatory network of tumor-derived cytokines regulates immune surveillance in PDA particularly with regard to macrophages and other myeloid cells [42]. As recently reported, mesenchymal cells in the PDA tumor microenvironment, such as FAP^+ cells, may also regulate tumor immunity [44].

Summary

In summary, activation of the CD40 pathway is a promising and novel therapy for PDA. CD40 antibodies have achieved significant and durable responses in patients with a wide range of tumor histologies, including PDA. CD40 immunotherapy can drive both T-cell-dependent and T-cell-independent mechanisms of action, and we believe the latter, particularly in PDA, is linked to the re-education of tumor-promoting macrophages and stromal involution. Finally, T-cell responses against this tumor appear limited by the inhibitory mechanisms of the tumor microenvironment, and future work is geared toward understanding how blockade of these inhibitory mechanisms may enable adequate priming of an adaptive immune response in concert with CD40 activation. Modulation of the immune microenvironment of the tumor may represent a useful approach in cancer immunotherapy.

Acknowledgments We thank Ben Stanger, Andrew Rhim, Anil Rustgi, Peter O'Dwyer, and Ursina Teitelbaum for helpful discussions. This work was supported by grants from the National Institutes of Health (R01 CA158186 and R01 CA169123, to Robert H. Vonderheide; T32 HL007775 to David L Bajor; and K08 CA138907 to Gregory L Beatty).

Conflict of interest The authors declare that they have no conflicts of interest to disclose.

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