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Harnessing B cells for cancer immunotherapy.

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Abstract

B cells are a heterogeneous population in immune defense system with multidirectional functions. In cancer patients, B cell infiltrates are associated with a significant increase of overall survival. We have recently developed a GM-CSF and IL-4 derived fusion cytokine named GIFT4, which has the capability to prime naïve B cells into anti-tumor immune effector cells. Herein, we overview current research findings on B cell anti-tumor functions and B cell-based approaches for cancer immunotherapy. We predict that GIFT4-augmented B cells as a potent cellular therapeutic could provide a new approach for cancer immunotherapy.

Keywords: B cells, Tumor microenvironment, GIFT4 fusokine, Cancer immunotherapy.

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Introduction

B Cell Functions

B cells are one of the two adaptive arms partnering with T cells in immune defense system against infections by virus, bacteria, fungi and parasites [1]. Originated from hematopoietic stem cells in bone marrow, B cells contain multiple subsets including antibody-secreting cells, antigen-presenting cells (APC), innate B effector cells and regulatory B cells [2]. As a heterogeneous population, B cells possess multidirectional immune functions. For instance, B cells can produce antigen-specific antibodies in response to infectious pathogens or sterile self-antigens [3]. B cells can also present pathogen-derived antigens to T cells during infections [4]. B effector cells can further produce a variety of immune-stimulatory cytokines such as IL-1, IL-6, IL-12 [5], Granulocyte-macrophage colony-stimulating factor (GM-CSF) [6], augment immune response against infections or promote inflammation in autoimmune diseases [6,7]. In contrast, regulatory B cells secrete immune-suppressive cytokines including IL-10 and transforming growth factor-β (TGF-β) to attenuate pro-inflammatory immune response [8]. Emerging evidences show that B cells also have anti-tumor function [9,10]. In preclinical animal model, B cells are required for the successful combined antibody-immunotherapy against murine mesotheliomas [11]. In patients with malignancies, B cells are also found to correlate with a significant increase of overall survival, and higher number of B cell infiltrates lead to better prognosis [12]. However, B cells in particular regulatory B cells can also act as immune-suppressive cells and facilitate tumor immune escape [13,14]. The dual functional faces of B cells on tumors are likely due to the different B cell subpopulations, which have distinguished phenotypes and secretomes that either inhibit tumor growth or facilitate malignancy [9].

B Cells in Tumor Microenvironment

B cells as well as T cells, natural killer cells, monocytes and other immune cells can infiltrate into tumor microenvironment, distributing from the tumor margin to the tumor core. In patients with tongue squamous cell carcinoma, infiltrated B cells are commonly found in the carcinoma stroma with tumor-suppressive effect [12]. In pancreatic ductal adenocarcinoma, human B cells reside in tertiary lymphoid tissue with two distinct infiltrating patterns: scattered or organized [15,16]. High density of organized infiltrating B cells predicts longer survival for patients; highlighting B cells are essential effector cells in the tumor microenvironment of human pancreatic ductal adenocarcinoma [16]. In bladder cancer, human $CD20^+$ B cells preferentially migrate into the lamina propria area, and have positive correlation with T cell infiltration [17]. Further analyses demonstrated that B cell infiltrates have no link with Foxp3 positive regulatory T cells in tumor microenvironment [17]. Moreover, tertiary lymphoid structures with aggregating B cells are associated with lung cancer prognosis [18]. In patients with gastric cancer, B cells abundantly infiltrate and aggregate in the gastric cancer stromal microenvironment, accompanied with infiltrated T-bet^+ T cells to form a tertiary lymphoid structure surrounding the tumor [19]. Tumor-associated B cells in gastric cancer microenvironment are proliferating and express Ki67. Importantly, infiltrated B cell number

is positively linked to relapse-free survival, and B-cell gene expression is significantly connected with improved outcome [19]. It is found that tumor-infiltrating B cells have beneficial effects on prognosis in patients with tongue squamous cell carcinoma [12], pancreatic adenocarcinoma [16], gastric cancer [19], cutaneous melanoma [20,21], breast cancer [22], ovarian cancer [23] and colorectal cancer [24]. However, the mechanisms by which B cells accumulate in the tumor microenvironment and result in better prognosis are not fully understood. One explanation is that tumor-infiltrating B cells express antigen-presentation molecules and function as professional APC to orchestrate T cell-mediated anti-cancer immunity [25,26]. Another reason could be that infiltrated B cells have potent capacity to produce anti-tumor antigen-specific antibodies, since $CD138^+$ and immunoglobulin kappa C-positive plasma cells have positive impact on anti-tumor immunity and are related to favorable prognosis in cancer patients [24,27]. MUC1 (The polymorphic epithelial mucin) is one of the most specific tumor-associated antigens in human cancers [24,28]. Anti-MUC1 IgG antibodies but not IgM in patients are significantly related to better prognosis [22]. Consistently, high density of plasma cells was found surrounding the tertiary lymphoid structures and correlated to T cell cytotoxicity [29]. Infiltrating B cells can also undergo somatic mutation, clonal expansion, intraclonal variation and isotype switching, eliciting humoral immunity against tumors [30,31]. Collectively, the preclinical and clinical investigations strongly support the notion that B cell infiltrates in the tumor microenvironment not only serve as a valuable predictive biomarker, but also play a profound protective role in anti-tumor immunity [32-35].

Cancer Immunotherapy

During the last decade, great progress has been made on cancer immunotherapy including dendritic cell-based cell immunotherapy [36], chimeric antigen receptor (CAR)-T cell [37,38] and immune checkpoint blockade including CTLA-4 (Cytotoxic T-lymphocyte-associated protein 4) or PD-1 (Programmed cell death protein 1)/ PD-L1 (Programmed death-ligand 1) inhibitors [39-41]. Dendritic cells as the most professional APC possess the capability to orchestrate innate and adaptive cellular and humoral immune responses against cancer cells. Cancer-antigen loaded or bioengineered dendritic cells that expressing tumor antigens have been utilized as cancer vaccines for cancer patients [42]. However dendritic cells as a tumor vaccine in clinical trials are not as effective as in preclinical animal tumor models, with the limitation of high-cost, small number and short life (2-3 days after maturation) of dendritic cells generated from peripheral blood monocytes. CAR-T cells have been successfully used to treat B-cell malignancies by targeting CD19, CD20, CD22, CD30, CD33, CD123, CD133, CD138, ROR1, κ light chain and B-cell maturation antigen [43]. The killing of normal B cells besides malignant B cells by CAR-T cells and its serious treatment-related toxicities remains a challenge [44]. Current clinical trials reveal that CAR-T therapy have very limited efficacy on nonhematological solid tumors. Expression of regulatory molecules such as CTLA-4 and PD-1 on cytotoxic T cells has been shown to suppress the anti-tumor functions of T cells. Thus immune checkpoint blockade using antagonistic antibodies against the negative regulators can overcome cancer immune resistance and demonstrates promising therapeutic efficacy [45-47]. However, clinical trials showed that only partial cancer patients respond to immune checkpoint blockade [48,49]. B cells have multiple functions as antibody-producing cells; antigen-presenting cells, immune effector cells, and are required for adaptive T cell immune responses against tumors [50]. B cells also have an advantage to be easily expanded *ex vivo* in comparison with dendritic cells. Moreover, activated B cells can effectively present tumor lysate, antigen peptide or antigen cDNA and induce antigen-specific T cell immunoreaction against tumors [51]. Thus, B cells represent a promising approach for cancer immunotherapy, complementing the use of dendritic cells.

B Cell Based Approaches for Cancer Immunotherapy

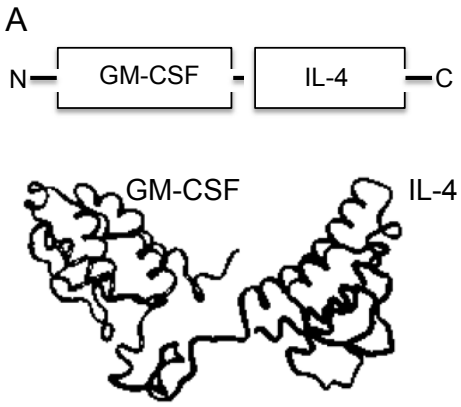
B cells have been widely explored as a cellular adjuvant for cancer immunotherapy due to its immune-stimulatory activities. As antigen-presenting cells, B cells express CD40 and ligation with CD40 ligand on B cells robustly enhances the expression of co-stimulatory molecules CD80 and CD86 [52]. Consequently, CD40-activated B cells have potent capability to promote naïve and memory T activation and expansion and induce cytotoxic T cells immunity [53]. When pulsed with a melanoma antigen, CD40-activated B cells efficiently propel the generation of melanoma-specific T cells *in vitro* [54]. CD40-activated B cells also express adhesion molecules and chemokine receptors facilitating the cells to migrate into the secondary lymphoid organs, attract and interact with antigen-specific T cells [52-55]. CD40-activated B cells also function similarly to plasma cells and produce IgG [52]. *In vivo*, CD40-activated B cells have protective effect on various tumor models [56,57], with little toxicity to the mice [56]. Alternatively, CD40-ligated B cells loaded with tumor-specific RNA as a cancer vaccine induce tumor-specific cytotoxic T cell immune response, inhibit the growth of non-Hodgkin's lymphoma and improve overall survival in preclinical animal model [57]. It is interesting that leukemia B cells activated by CD40 ligation are also functionally similar to antigen-presenting cells and induce both IFN-γ^+ CD4 and cytotoxic CD8 T cell proliferating and expansion [58]. Those data together inform that CD40-activated B cells have the potential to serve as a potent cellular agent for cancer immunotherapy.

Tumor-infiltrated B cells provide another approach for B cell cancer immunotherapy. B cells infiltrated into tumor stroma function as both antigen-presenting cells and tumor antigen-specific antibody-producing cells,

and play essential roles in anti-tumor immunity [34,59]. A Epstein-Barr virus immortalization *in vitro* assay demonstrates that primary colorectal carcinoma harbor infiltrated B cells that are consistent of $CD23^{+}CD80^{+}$ activated antigen-presenting cells and IgG-secreting cells. Those infiltrated B cells not only produce functional carcinoma-specific antibodies [59], but are also associated with cytolytic T cell response and superior prognosis in cancers [21,22,24,29]. Adoptive transfer of tumor-derived B cells further promotes anti-tumor T cell immunity and leads to tumor regression in preclinical breast cancer and pulmonary metastatic tumor animal models [60,61]. The anti-tumor property of tumor-primed B cells suggests that *ex vivo* expanded tumor-primed B cells could be utilized as potent T helper cells for cancer immunotherapy. B cells loaded with tumor-derived autophagosomes have the ability to present tumor-specific antigens selectively captured by autophagosomes and induce robust anti-tumor T cell response as well as antibody-mediated humoral response [62]. Administration of tumor-antigen loaded B cells as a vaccine further prevents the growth of tumors in mice [62], indicating that B cells activated by tumor-derived autophagosomes represent a new strategy for cancer immunotherapy.

Recently, we have developed an immune-stimulatory fusion cytokine (Fusokine) named GIFT4 (Figure 1), which is a granulocyte macrophage colony-stimulating factor (GM-CSF) and common γ-chain Interleukins 4 (IL-4) fusion transgene [63]. In comparison with its parental cytokines, GIFT4 fusokine gains new function distinct from its parental cytokines GM-CSF and IL-4. GIFT4 has potent capability to activate and program naive B cells into immune effector cells. Programming of naïve B cells by GIFT4 fusokine involves both GM-CSF and IL-4 domains through a synergistic recruitment of GM-CSF receptor and IL-4 receptor clustered on B cell surface, which further triggers the formation of downstream signaling complex of JAK1 (The Janus kinase 1), 2, 3 and STAT1 (The signal transducer and activator of transcription 1), 3, 5 and 6 [63]. Inhibition of JAK signaling by its specific inhibitors completely interrupted GIFT4-induced STAT1, STAT3, STAT5 and STAT6 signaling in the treated B cells and consequent B cell expansion. In contrast, combined use of parental cytokines GM-CSF and IL-4 is unable to cluster the two receptors on B cell surface and induce B cell proliferation.

Interesting, GIFT4 protein has no effect on monocytes, although GM-CSF and IL-4 together have the capability to promote monocytes differentiation into dendritic cells. GIFT4-augmented B cells (GIFT4-B cells) express co-stimulatory molecules CD40, CD80 and CD86, and produce unique immune-stimulatory cytokines, chemokines and adhesion molecules including IL-1α, IL-6, IL-12, GM-CSF, CCL3, CCL4 and CD54, but little IL-10 and IFN-γ [63], apart from CD40-activated B cells [52] or innate response activator B cells [6]. With those immune properties, GIFT4-B cells function as APC-like effector cells, and consequently promote the expansion of $CD314^{+}$, granzyme B-, granulysin- and IFN--producing cytotoxic T cells that selectively kill human melanoma cells both *in vitro* and *in vivo* [63]. Moreover, GIFT4 fusokine induces B cell-dependent anti-tumor immunity in murine melanoma models [63], involving both APC-like B effector cells and GM-CSF-producing innate response activator B-cells [6,63]. In our investigation of GIFT4 as a potential vaccine adjuvant, we also discovered that GIFT4-coated virus-like particles enhance anti-HIV antigen-specific antibody production *in vivo* [64], suggesting additional effect of GIFT4 on the antibody-secreting cells. Indeed, we have found that administration of GIFT4 protein induces robust anti-melanoma specific-antibody production in murine melanoma model (Unpublished data). We have further extended our investigation to human chronic lymphocytic leukemic (CLL) B cells, and examined the immune activity of GIFT4-stimulated CLL B cells (GIFT4-CLL cells). Unlike CD40-activated CLL cells [58], TLR9 ligand-treated CLL cells [65] or normal GIFT4-B cells [63], GIFT4-CLL cells produce immune-stimulatory cytokines including IL-1β, IL-2, IL-6, IL-8, ICAM-1 and prime autologous T cells to proliferate, express tumor-killing molecules IFN-γ, CD314, perforin and granzyme B, and lyse autologous primary leukemic cells [66]. Taken together, GIFT4 induces broad anti-tumor B cell immune



B

MWLQSLLLLGTVACSISAPARSPSPSTQPWEHV
NAIQEARRLLNLSRDTAAEMNETVEVISEMFDLQ
EPTCLQTRLELYKQGLRGSLTKLKGPLTMMASH
YKQHCPPTPETSCATQTITFESFKENLKDFLLVIP
FDCWEPVQE**S**MGLTSQLLPPLFFLLACAGNFVH
GHKCDITLQEIIKTLNSLTEQKTLCTELTVTDIFAA
SKNTTEKETFCRAATVLRQFYSHHEKDTRCLGA
TAQQFHRHKQLIRFLKRLDRNLWGLAGLNSCPV
KEANQSTLENFLERLKTIMREKYSKCSS

Figure 1. *Structure of GIFT4 protein. (A) GIFT4 protein structure that contains GM-CSF and IL-4 domains. (B) Amino acids of human GIFT4*

responses either through GIFT4-programmed B effector cells that further prime tumor-killing cytotoxic T cell response, or through the augmentation of tumor-specific antibody production. Those results provide a strong basis for the potential utilization of GIFT4 fusokine and GIFT4-augmented B cells as well as GIFT4-converted CLL cells for cancer immunotherapy in human.

Conclusion

B cells play pivotal roles in immune defense system, which bridge the innate and the adaptive immunities against cancers. Augmented B cells including GIFT4-B cells and expanded tumor-infiltrated B cells have potent immune-stimulatory activities and anti-tumor function by either priming cytotoxic T cell response or producing anti-tumor specific antibodies. We predict that GIFT4 and GIFT4-augmented B cells as potential immune therapeutics could provide a new approach for cancer immunotherapy.

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Disclosure

The authors declare that they have no competing interest.

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