Cancer Immunotherapy Targeting Cripto-1

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The immune system has been found to be suppressed in cancer patients. Cancer cells are extremely resistant to chemotherapeutic drugs, conventional immunotherapy, or cancer antigen vaccine therapy. Cancer immunotherapy, which is mainly based on immune checkpoint inhibitors, such as those for PD-1, PD-L1, and CTLA4, is an effective treatment method. However, no immunotherapeutic target has been found that retains validity in the face of tumor diversity. The transforming growth factor (TGF)-β cytokine family possesses broad biological activity and is involved in the induction and/or transdifferentiation of helper T cells, which are important in immunotherapy. Nodal is a member of the TGF-β family playing important roles in tissue stem cells and cancer stem cells (CSCs), interacting with the co-receptor Cripto-1, as well as with Activin type IB (Alk4) and Activin typeIIreceptors, and maintaining stemness and Notch and Wnt/β-catenin signaling in CSCs.

Keywords: Cripto-1 ; TGF-β ; cancer stem cells ; immunotherapy ; antibody

II1. Introduction

Since van der Bruggen et al. first identified a human melanoma antigen recognized by cytolytic T cells in 1991 [1], tumor immunogenicity has been studied by many researchers as a potential feature to target in cancer treatment [2]. Tumor immunogenicity is responsible for the immune responses against cancer, activated as a host defense mechanism through antibody production and cell-mediated immunity. However, tumor immunogenicity varies depending on the type of cancer. Immunogenicity can be altered by mutations in tumors, which may in turn function as immunostimulants to induce cytokines or interferons. In this case, the concept of cancer vaccines is valid and has been developed in the past to establish cancer immunotherapy. The initial strategies were aimed at boosting the immune system that attacks cancer, but the therapeutic effect was not satisfactory on every patient. To accomplish a more robust response, research in cancer immunotherapy has been focused on T cell activation. T cells can attack cancer antigens that are presented on the cell surface. Immune checkpoint inhibitors (ICIs), such as programmed cell death 1 (PD-1) [3][4], programmed death-ligand 1 (PD-L1) [5][6], and cytotoxic T-lymphocyte antigen 4 (CTLA-4) [2][7][8], have been developed as effective agents in cancer immunotherapy. To enhance T cell activation, ICIs inhibit the interaction of T cells and cancer cells mediated by PD-1 and PD-L1, which enables cancer cells to escape from T-cell attack; therefore, ICIs' mechanism of action is different from that of conventional cancer immunotherapy that directly boosts immunity as a method by which to attack cancer cells. Upon binding of PD-L1 to PD-1, signaling is transduced in T cells involving SHP2 to inhibit the proliferation and differentiation of T cells previously activated by a tumor antigen presented by antigen-presenting cells (APCs) through MHC. Therefore, ICIs allow activated T cells to recognize and attack tumor cells [9][10]. The successful therapeutic effect of ICIs is controversial, because only around 5–30% of all cancer patients who receive this therapy effectively respond to it. This response is not as good as that to conventional anticancer drugs, including monoclonal antibodies that target cancer-specific antigens. Therefore, other types of cancer immunotherapies are being developed [11].

The transforming growth factor β (TGF-β) family is a cytokine family with broad biological activity, involved in the induction of proliferation and/or differentiation of cells. Helper T cells, which are important in immunotherapy, are transdifferentiated by TGF-β, while TGF-β inhibits the differentiation of cytotoxic T lymphocytes (CTLs). TGF-β promotes the development of peripheral tumor-resident regulatory T (pTreg), Th17, Th9, and T follicular helper (Tfh) cells [12]. Cancer growth is regulated by various cytokines, including TGF-β, that are produced in the tumor microenvironment [13]. In recent years, Tauriello et al. reported that inhibition of TGF-β causes a strong and persistent cytotoxic T cell response against tumor cells, which prevents metastasis. They showed that increased TGF-β in the tumor microenvironment is a major mechanism of immune avoidance by promoting T cell elimination and blocking the acquisition of the T helper 1 (Th1) effector phenotype in advanced colorectal cancer [14]. Mariathasan et al. reported that enhanced TGF-β signaling in patients' fibroblasts reduced patients' responses to treatment in a cohort study analyzing specimens from patients with metastatic urothelial cancer. These authors also showed that the therapeutic co-administration of anti-TGF-β antibody and

anti-PD-L1 antibody reduced TGF-β signaling in stromal cells and promoted T cell penetration into the center of the tumor [15].

Cripto-1 is a developmental oncoprotein highly expressed in many cancer cells, where it promotes tumorigenesis and is essential in embryogenesis. Cripto-1 is known as a co-receptor for Nodal, a member of the TGF-β family [16][17]. Cripto-1 is necessary for Nodal to bind to the Alk4 activin type I receptor. Several studies have shown that Cripto-1 plays an important role in early embryonic development as a co-receptor for Nodal [18][19]. Recently, Cripto-1 has been shown to be involved in the maintenance of cancer stem cells (CSCs) in various cancers [20][21][22][23][24][25].

2. Cripto-1 as a Novel Target for Immunotherapy

CSCs are present in most tumors. CSCs are characterized by their ability for self-renewal and differentiation, which maintains their original phenotype and, at the same time, allows the production of more differentiated cells within a tumor. CSCs are dormant and resistant to drug and radiation treatments, which leads to tumor recurrence and metastasis [26][27][28][29]. From this perspective, it is important to target CSCs in cancer therapy.

TGF-β has been shown to be an effective target in immunotherapy for cancer treatment. For example, two recent studies have found that TGF-β suppresses the antitumor immune response by inhibiting the infiltration of T cells into tumors [14][15]. On the other hand, Treg cells, which contribute to the inhibition of antitumor immune responses, were shown to harbor a latent form of TGF-β on the cell surface [30]. TGF-β has multiple functions in CSCs and can either inhibit or maintain various CSC traits [31]. TGF-β suppresses tumorigenesis by promoting the differentiation of CSCs, downregulating the expression of ABCG2, which acts as a transmembrane transporter, inducing chemoresistance in CSCs, and decreasing the aldehyde dehydrogenase 1 (ALDH1)-positive population, which is capable of self-renewal, as well as of enhancing tumor initiation/progression through CSCs. On the other hand, TGF-β promotes the stemness of CSCs in malignancy, induces the self-renewal of CSCs, and negatively regulates DNA methyltransferases, enhancing tumorigenesis. Oshimori et al. found that TGF-β in squamous cell carcinoma cells can significantly enhance glutathione metabolism and reduce the effectiveness of anticancer treatments by transcriptionally activating p21, which stabilizes NRF2 [32]. TGF-β can be a positive or negative regulator of cancer immunity [33]. Therefore, other TGF-β-related cytokines may be more convenient targets for immunotherapy.

Cripto-1 is a unique co-receptor required for Nodal activity. Blocking Cripto-1 would be a potential approach to inhibit Nodal, which is expressed in a number of different cancers [34][35] and which can enhance tumor growth and metastasis. Cripto is anchored by a glycosylphosphatidylinositol (GPI) motif to the surface of undifferentiated cells, including CSCs [36]. Cripto-1 contains EGF and CFC motifs which bind to Nodal and Alk4, respectively. Cripto-1 may be a suitable target for monoclonal antibody and vaccine therapies. Ligtenberg et al. and Witt et al. demonstrated that Cripto-1 vaccination was effective in inducing protective immune responses against metastases produced by sphere-forming CSCs of melanoma and mammary carcinoma cells [37][38]. In particular, in the study on melanoma, vaccination elicited a cytotoxic $CD8^+$ T cell response. Another research group investigated the effect of Cripto-1 overexpression on macrophage activity and the underlying mechanism [39]. This study suggested that Cripto-1 enhances macrophage phagocytic activity and upregulates the production of anti-inflammatory and proinflammatory cytokines via the NF-κB signaling pathway. Stifter et al. investigated the effect of transporter-mediated tumor antigen expression associated with antigen processing on PD-1/PD-L1-CD8 T cell priming using pancreatic ductal adenocarcinoma cells (PDACCs) in mice deficient for PD-1/PD-L1-competence [40]. They identified Cripto-1 as one of the two antigens that were expressed in the endoplasmic reticulum of PDACCs.

Cripto-1 has been shown to be highly expressed not only in embryonic stem cells but also in tumors where it promotes tumorigenesis [17][22], suggesting that it promotes the maintenance of a stem cell phenotype. Cripto-1 is involved in breast cancer tumorigenesis and is beginning to attract attention as a novel target for the treatment and diagnosis of breast cancer [41]. The simultaneous targeting of Nodal and Cripto-1 proteins has been shown to have therapeutic potential for oral squamous cell carcinoma [42]. Suppression of Cripto-1 expression inhibits cell stemness and EMT-related gene expression, significantly reducing self-renewal capacity, tumorigenesis, and metastasis in esophageal squamous cell carcinomas (ESCCs). Cripto-1 has also been demonstrated to regulate the Wnt/β-catenin signaling cascade and promote cell proliferation, migration, and invasion in hepatocellular carcinoma (HCC) [23]. Cripto-1 enhanced self-renewal capacity and conferred chemoresistance in HCC cells. Alowaidi et al. investigated the effect of Cripto-1 on pathways that control glioblastoma (GBM) cell function using phospho-specific protein microarray analysis, which suggested that angiogenesis could be mediated by Cripto-1 and that Cripto-1 might regulate the motility and infiltration of cancer cells [43][44]. Thus, Cripto-1 has the potential to be useful as a predictive and diagnostic marker in a therapeutic context (Table 1).

Table 1. Recent research papers on Cripto-1 as a therapeutic target.

Organ	Cancer Cells	CSCs
Breast	Regulation of human Cripto-1 expression by nuclear receptors and DNA promoter methylation in human embryonal and breast cancer cells [45]	Cripto-1 Plasmid DNA Vaccination Targets Metastasis and Cancer Stem Cells in Murine Mammary Carcinoma [38]
	Cripto-1 as a novel therapeutic target for triple-negative breast cancer [46]	
Brain	Cripto-1 overexpression in U87 glioblastoma cells activates MAPK, focal adhesion, and ErbB pathways [43]	
	Investigating the role of CRIPTO-1 (TDGF-1) in glioblastoma multiforme U87 cell line [47]	
	Cripto-1 localizes to dynamic and shed filopodia associated with cellular migration in glioblastoma cells [48]	
The others	Overexpression levels of cripto-1 predict poor prognosis in patients with prostate cancer following radical prostatectomy [49]	Cripto-1 acts as a functional marker of cancer stem-like cells and predicts prognosis of the patients in esophageal squamous cell c[6][7][8][9][10][11][12][13][14][15][16][17][18][19][20]arcinoma [25]
	Expression and functional role of CRIPTO-1 in cutaneous melanoma [50]	Cripto-1 contributes to stemness in hepatocellular carcinoma by stabilizing Dishevelled-3 and activating Wnt/β-catenin pathway [23]
	The role of Nodal and Cripto-1 in human oral squamous cell carcinoma [42]	Exogenous Cripto-1 Suppresses Self-Renewal of Cancer Stem Cell Model [51]
	CRIPTO promotes an aggressive tumor phenotype and resistance to treatment in hepatocellular carcinoma [52]	Dynamic regulation of the cancer stem cell compartment by Cripto-1 in colorectal cancer [24]

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