Comment

Co-signaling surface receptors: regulators of adaptive immune response. Comment on Shen H, *et al.* "Co-signaling receptors regulate T-cell plasticity and immune tolerance". Frontiers in Bioscience-Landmark. 2019; 24: 96–132

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It is well-established that adaptive immunity is initiated when the T lymphocytes through the T cell receptor (TCR) recognize specifically the antigenic peptide in the context of major histocompatibility complex (MHC) expressed on professional antigen presenting cells (APC) such as dendritic cells (DC) or virus-infected epithelial cells [1–3]. TCR recognizes the peptide specific antigen associated with a polymorphic region of MHC while either CD8 or CD4 molecules, linking to a monomorphic region of MHC class-I or class-II antigens respectively, contribute to the stabilization of this interaction and cooperate to the TCR-CD3 complex-mediated signal transduction [1–3].

To give a full activation of T lymphocytes through TCR-CD3 complex, a second signal is necessary and the discovery of the prototype co-signaling molecule CD28, interacting with B7-1 (CD80) or B7-2 (CD86) on APC, confirmed this notion [4, 5]. Following the CD28 receptor, a plethora of co-signaling molecules have been identified [6]. Furthermore, the discovery of co-inhibitory receptors such as the cytotoxic T lymphocyte antigen (CTLA) 4 and programmed death receptor (PD) 1 revealed that the T lymphocyte activation can be regulated by surface molecules that down-modulate the TCR-initiated signal [5, 6]. Importantly, CD28 prefers the binding of monomeric CD80 and CD86 while CTLA-4 favors that of dimeric ligands, suggesting that the ratio of CD80 and CD86 in monomeric versus multimeric forms could affect the immune response of naïve T cells [7]. This finding would strengthen the relevance of the structural protein association of ligands expressed on APC for co-signaling molecules in triggering T

cells. Several co-stimulating or co-inhibiting receptors of the TCR signalling have been identified in the last years [6–8] opening the era of the therapeutic use of blocking antibodies of co-inhibitory receptors to raise the activities of T lymphocytes [9–11]. Of course, the use of drugs modulating the function of co-signaling molecules can be applied with opposite goals [12]. Indeed, in autoimmune diseases and allotransplantation the aim is to block the host immune response while in cancers the immune response against self-cells should be triggered [12]. Of note, the administration of immune checkpoint blockers led to relevant clinical successes for the therapy of some solid tumors such as non-small cell lung carcinoma (NSCLC) and melanoma [9–11]. Indeed, first anti-CTLA4 antibody ipilimumab and later on the anti-PD1 antibody nivolumab have been the prototypes of very efficient drugs in treating incurable solid tumors with unexpected good clinical results [10–14]. These findings, with our progressive understanding of interplay of co-signaling receptors, have determined a revisitation of the original paradigm represented by the CD28-B7-1/2 interaction [3, 4]. Indeed, T lymphocyte through co-signaling receptors can sense the external microenvironment and respond accordingly [9–12, 15]. It is the complex interaction among co-stimulatory and/or co-inhibitory receptors and their corresponding ligands on APC, endothelial cells (EC), vascular smooth muscle cells (VSMC), stromal cells and other cells present in the microenvironment that will determine whether T lymphocytes will be able to grow, differentiate, exert different functional activities, survive and eventually die. In this context, it is evident that also the mi-

croenvironment will respond following the interaction with T lymphocytes [6]. Thus, the cells in the microenvironment may influence the expression of several co-signaling molecules and, by consequence, the immune response both in health and disease [15]. Indeed, key issues unresolved are the molecular mechanisms of regulation of expression of co-stimulatory and/or co-inhibitory molecules and how the intracellular pathways involved in the co-signaling are integrated to determine a well-defined and specific effect on T lymphocyte fate in association with the TCR-CD3 complex mediated signaling.

On this point, the paper published by Shen H. and colleagues [15] is of great interest because it faces (i) the expression profile of co-signaling receptors in humans under physiological conditions; (ii) whether some of these receptors are regulated in pathological situations such as cancer and inflammation; (iii) whether pro- and anti-inflammatory signals are associated with either up- or down-regulation of some of these receptors [15]. The authors have used a large microarray dataset of co-signaling molecules obtained with high-throughput functional genomics experiments from Array Express of European Bioinformatics Institute (https://www.ebi.ac.uk/arrayexpress). They have shown that co-signaling receptors are differentially expressed in healthy tissues; this expression changes in the tumor counterpart and in some instances a very strong expression of co-signaling receptors has been detected [15]; furthermore, the authors have shown that the defect of the signal transducer and activator of transcription (STAT) 1 [10, 15] triggers the up-regulation of MHC class II molecules and some co-stimulatory surface molecules [15, 16], suggesting that STAT1 can affect T lymphocyte plasticity and consequent immune response.

Focusing on the expression of co-stimulatory and co-inhibitory receptors in cancers, the authors have reported that co-inhibitory receptors are more expressed than co-stimulatory ones [15]. Of note the highly expressed co-inhibitory molecules were galectin 9, SEMA4A, B7-H3, B7-H4 and VISTA (B7-H5) [15]. These findings are in line with the well-established notion that the tumor microenvironment (TME) is immunosuppressive [17] and it would suggest the possibility to define the suitable targets to be blocked when using immune-checkpoint blockade therapy possibly in combination with anti-PD1 or anti-CTLA4 humanized antibody [14]. In this context, it is relevant to point out that the TME is a complex association of tumor cells, innate and adaptive immune cells, tumor associated fibroblasts (TAF), mesenchymal stromal cells (MSC) in different stages of differentiation, vascular and lymphatic endothelial cells [17]. This TME is locally heterogenous due to the different proportions of these cells and it is strongly influenced by the extracellular matrix components and cell-derived secretome [17]. Furthermore, the TME is highly dynamic triggering different signaling pathways during disease progression and/or as a consequence for therapeutic

regimen as it has been shown in the case of immunomodulatory drugs [18]. From this reason the characterization of co-signaling molecules on immune cells and the corresponding ligands in the TME are essential to plan the appropriate therapy [14, 15].

Importantly, the Authors have defined three different groups of tumors based on the prevalence of expression of (i) co-stimulatory or (ii) co-inhibitory receptors or (iii) moderate expression of both [15]. Of note, it appears that overall tumors have a lower content of co-stimulating molecules compared to healthy tissue counterpart suggesting that the downregulation of these receptors can be a mechanism by which cancers cells and TME try to elude T lymphocyte-mediated control. Importantly, a correlation between the increased co-stimulation molecules repertoire and the improved final prognosis can be detected together with a lower representation in the tumor of type 2 macrophages (M2) with an anti-inflammatory effect [15]. This observation agrees with several findings reported in the literature for a key role of this cell population in regulating anti-tumor immune response in cancers [19].

However, the results reported in the paper by Shen H. and colleagues should be confirmed and validated through different methodological approaches such as immunohistochemistry, multiparametric flow cytometry, quantitative polymerase chain reaction, western blotting, and depth analysis at the single cell level.

Nevertheless, the experimental approach of Shen H. and colleagues can be very useful to immediately understand the pattern of expression of the different co-stimulatory and co-inhibitory T cell surface receptors; this bioinformatic approach on the patient tumor biopsy can be associated with the generation of in vitro 3D cultures such as organoids or spheroids of patients-derived tumor cells: these 3D cultures can be used to test whether the targeting with specific drugs of co-inhibiting and/or co-activating receptors can influence tumor cell growth and immune response [20]; eventually, the results of these tests may suggest the more efficient combinations of the several tools able to wake up the anti-tumor immune response to try and control tumor cell growth and expansion.

1. Ethics approval and consent to participate

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4. Conflict of interest

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