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REVIEW ARTICLE

CMTM6 as a master regulator of PD-L1

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Abstract
Immune checkpoint proteins, such as programmed cell death receptor 1 (PD-1) and its ligand (PD-L1), play critical roles in the pathology of chronic inflammatory pathological conditions, particularly cancer. In addition, the activation of PD-1/PD-L1 pathway is involved in mediating resistance to certain anti-cancer chemo- and immuno-therapeutics. Unfortunately, targeting the PD-1/PD-L1 pathway by the available anti-PD-1/PD-L1 drugs can benefit only a small proportion of cancer patients. Thus, studying the factors that regulate the expression of these immune checkpoint proteins is of central importance in this context. Recent investigations have identified CMTM6 and, to a lesser extent, CMTM4, as master regulators of PD-L1 expression in various cancer cells. Understanding the mechanisms by which such proteins upregulate the expression of PD-L1 in tumor cells, and determining the potential regulators of CMTM6 expression in different types of cancers will accelerate the development of new therapeutic targets and/or lead to the enhancement of the currently available PD-1/PD-L1 blockade therapies.

Keywords Circular RNAs (circRNAs) · Hu-antigen R (HuR) protein · WEE1 and ATM Kinases · Epithelial to mesenchymal transition (EMT) transcription factor SNAIL · Endosomal degradation · Proteasomal degradation

Introduction

The immune system plays an indispensable role in fighting and clearing of abnormal cells including tumor/cancerous cells which exhibit uncontrolled proliferation. However, tumor cells can evade and resist killing mediated by the immune system through different mechanisms. One such mechanism is through increasing the expression of immune suppressor (immune checkpoint) proteins such as programmed cell death ligand-1 (PD-L1 also known as B7-H1, CD274 or PDCD1L1) on tumor cells [1–6]. The interaction between PD-L1 on tumor cells with its receptor, programmed cell death-1 (PD-1; also known as CD279, or PDCD1) protein, on T cells can suppress the activation of antigen-specific T cells and prevent the expansion of effector T cells. This results in a decrease in the anti-tumor immune responses mediated by T cells which, in turn, leads to an enhancement in the proliferative capacity of tumor cells leading to disease progression (Fig. 1) [3, 5]. PD-1 is an inhibitory receptor of the CD28 receptor family which belongs to the type I transmembrane proteins family. PD-1 plays a vital role in mediation of central and peripheral immune tolerance as well as immune exhaustion [7–11]. PD-L1 and PD-L2 are two ligands that bind to PD-1, and although a stronger binding affinity exists between PD-1 and PD-L2 in comparison with PD-1 and PD-L1, PD-L1 is considered as the primary ligand for PD-1 [12]. Thus, the focus of this review will be on PD-L1. Mutations in PD-1 have been associated with disease progression in different autoimmune disorders in humans, characterized by an abnormally increased immune activation against self-antigens, suggesting the inhibitory function of this receptor [13–15]. It has also been shown that knocking out this receptor in mice results in hyperactive immune responses [7, 16. Furthermore, expression of high levels of PD-1 on $CD8^+$ T cells has been linked with immune "$CD8^+$ T cells" exhaustion during chronic viral infections and a wide variety of cancer types [6, 10, 11, 17–19]. As previously mentioned, PD-1 expression has been linked with poorly functional/exhausted tumor-infiltrating immune cells in different cancer types [20–23]. Exhausted $CD8^+$ T cells are characterized by their inability to: (1) proliferate normally; (2) perform immune effector

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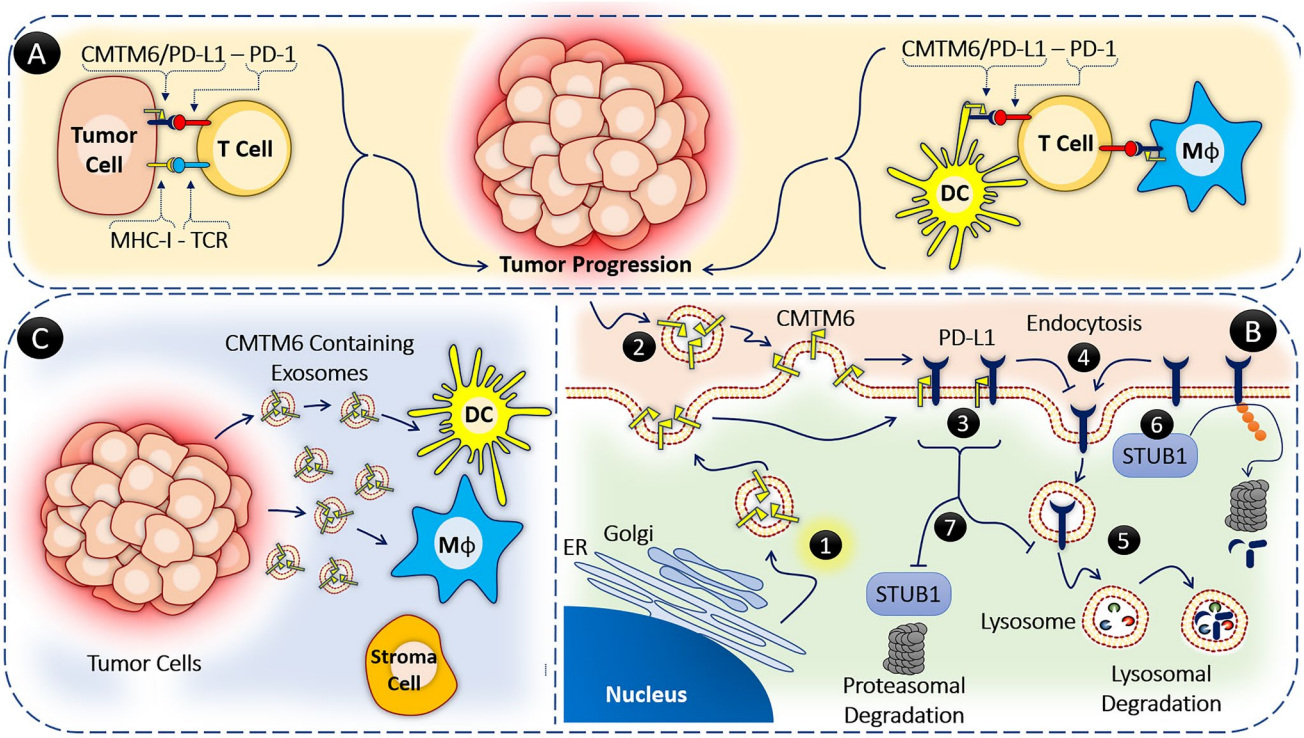

Fig. 1 CMTM6 and PD-L1 stabilization in tumor cells. **a** Inhibition of anti-tumor immunity mediated by effector T cells, which can be achieved in part through activating the PD-1/PD-L1 axis, facilitates tumor progression. CMTM6 protein can stabilize the expression of PD-L1 on both tumor as well as tumor stromal cells including antigen presenting cells such as macrophages (Mϕ) and dendritic cells (DC). **b** Represents the mechanisms of PD-L1 stabilization by CMTM6. Expression of CMTM6 could be induced in a cell as a result to an external or internal stimulation [1], and/or acquired from adjacent "tumor" cells through exosomes [2]. Indeed, recent investigations have confirmed that CMTM6 can stabilize the expression of PD-L1 at the intact cell surface [3]. In the absence of CMTM6, PD-L1 tend to be endocytosed for recycling and degradation [4]. One of the mechanisms by which CMTM6 can stabilize the surface expression of PD-L1 is through inhibiting the endosomal degradation during endosome recycling [5]. Ubiquitination of the PD-L1 is also another strategy that drives the downregulation of PD-L1 expression on cell surface through activating the proteasomal degradation pathway [6]. Interestingly, CMTM6 can also inhibit ubiquitination of PD-L1 through STUB1 (an E3 ubiquitin ligase), and thus it can decrease the degradation of PD-L1 through proteasomal degradation pathway [7]. **c** Shuttling CMTM6 through exosomes is considered as a potential strategy by which tumor cells could increase the surface expression of PD-L1 in tumor stroma cells including immune cells such as antigen presenting cells (dendritic cells "DC" and macrophages "Mφ") [96].

function; and/or (3) secrete normal amounts of cytokines [24–27], all of which are beneficial to cancer cells which will be able to resist the killing mediated by effector T cells.

Overexpression of PD-L-1 on tumor cells has been recently reported to increase resistance to chemotherapy, ionizing radiation, and immunotherapy [1, 2, 28–30]. For instance, PD-L1-expressing myeloma cells are associated with aggressive myeloma behavior (i.e., confer a proliferative advantage/resistance to anti-myeloma chemotherapy) [1]. Knocking down the expression of PD-L1 in myeloma cells significantly inhibited cell proliferation and increased apoptosis induced by the chemotherapeutic alkylating agent melphalan. This, in turn, strongly supports the importance of blocking the PD-1/PD- L1 axis [1, 3, 4]. In another study, Zhang et al. [2] assessed the association of PD-L1 expression with the response to cisplatin-based neo-adjuvant chemotherapy (NAC) and found an increased expression of PD-L1 in chemo-resistant tumors compared with chemo-sensitive tumors. They also reported that cisplatin can induce the upregulation of PD-L1 expression on non-small lung cancer cells, which is consistent with the recent findings that certain anti-cancer drugs can increase PD-L1 expression on tumor cells [2, 28]. However, the mechanism(s) by which cisplatin induces the expression of PD-L1 remain(s) to be determined. In line with these results, Jin et al. [29] have recently reported that resistance to trastuzumab, a monoclonal IgG1 antibody used against cancer cells (mainly breast and gastric cancer cells) which overexpress human epidermal growth factor receptor 2 (HER2), is due to the increased expression of PD-L1 on tumor cells, and this resistance can be reversed by blocking PD-L1.

Accordingly, the PD-1/PD-L1 axis not only plays a role in the pathogenesis of cancer, but also is crucial in

mediating resistance to cancer therapeutics. Hence, it is not surprising that blocking the activation pathway of PD-1/PD-L1 axis using the FDA approved anti-PD-1 (nivolumab, pembrolizumab, and cemiplimab-rwlc) and anti-PD-L1 (durvalumab, avelumab, and atezolizumab) monoclonal antibodies can enhance anti-tumor immune responses and benefit patients with different types of cancers. These cancers include, but are not limited to, relapsed or refractory Hodgkin's lymphoma, metastatic bladder cancer, non-small-cell lung cancer, advanced renal-cell carcinoma, advanced Merkel-cell carcinoma, and recurrent squamous-cell carcinoma of the head and neck [31–39]. Nevertheless, it is of particular importance to remember that only a fraction of cancer patients benefits from these treatments [40]. At the clinical level, the rate of successful response to PD-1/PD-L1 blockade therapy in cancer patients varies between low and moderate responses, but still, in certain cancer types, e.g., melanoma, the response rate may reach up to 45%, which is considered a relatively high response rate [36, 41–46]. This may be referred, at least in part, to the ability of cancer cells to exploit mechanisms other than the PD-1/PD-L1 pathway, in their resistance to the killing mediated by T cells (Fig. 2) [47]. Alternatively, certain mutations could render cancer cells resistant to anti-PD1/PD-L1 immunotherapy in cancer patients. For example, gene mutations in Janus kinase 1/2 (JAK1/2) that lead to loss of interferon gamma signaling are known to contribute to unresponsiveness to anti-PD1/PD-L1 immunotherapy [48]. This is because of the fact that JAK1/2 plays a central role in the signaling pathway of interferon gamma (IFN-γ) upon interaction with interferon gamma

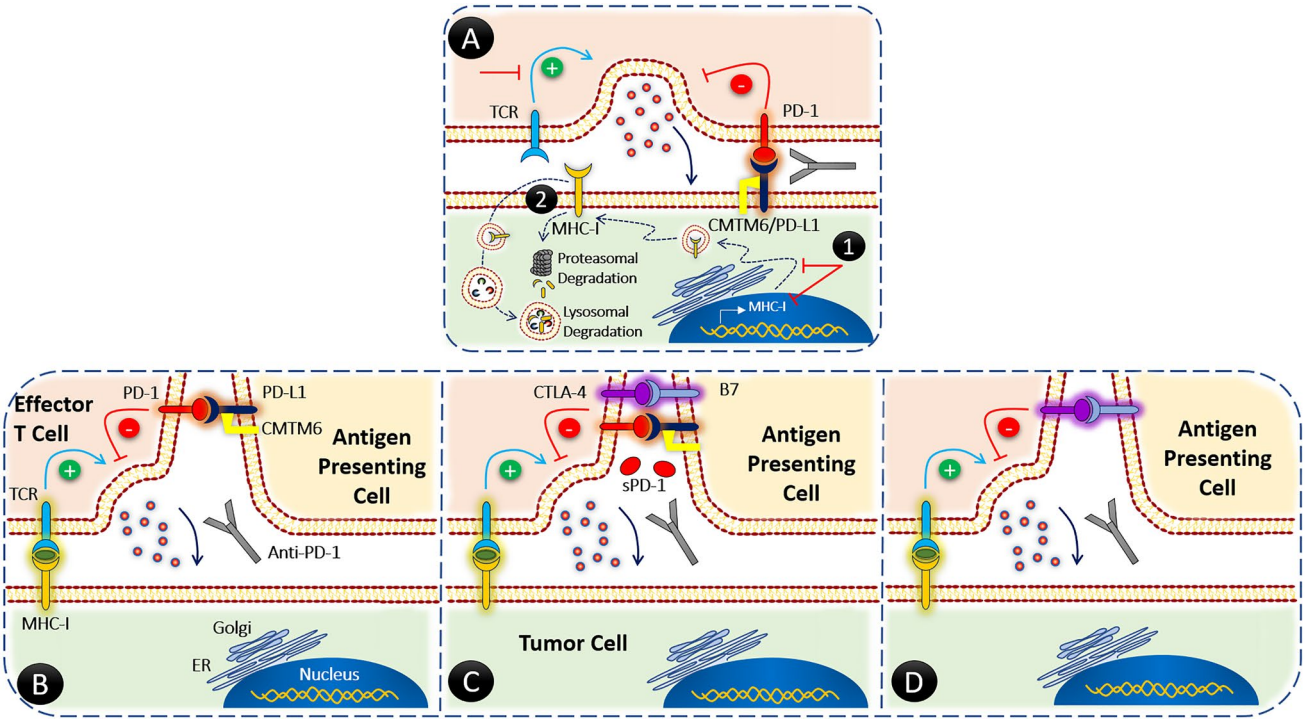

Fig. 2 Factors that affect the benefit to PD-1/PD-L1 blockade therapeutics. a The lack of or the low expression of major histocompatibility complex class I (MHC-I) limits the benefit to immune checkpoint inhibitors. The activation signal (+) of anti-tumor immune responses mediated by an effector T cell upon the engagement of T cell receptor (TCR) with the (MHC-I) expressed on tumor cells is inhibited (−) by the engagement of PD-1 with its ligand (PD-L1), and as such blocking the axis of PD-1/PD-L1 by a specific antibody is supposed to work in this case. However, regardless the presence of immune checkpoint proteins and their ligands, the absence or the low expression level of MHC-I molecule in cancer cells is considered as a strategy to evade anti-tumor immune responses. In this case, targeting the PD-1/PD-L1 axis is not supposed to be of therapeutic value. **b–d** figures represent the impact of expression of other immune checkpoint proteins on the response to PD-1/PD-L1 blockade therapy. The activation signal (+) of anti-tumor immune responses mediated by an effector T cell upon engaging of T cell receptor (TCR) with the major histocompatibility complex class I (MHC-I) expressed on tumor cells is inhibited (−) by the engagement of PD-1 with its ligand (PD-L1), and as such blocking the axis of PD-1/PD-L1 by a specific antibody is supposed to work in this case as seen in case (**b**), especially, because PD-1/PD-L1 axis is the only/major inhibitory mechanism. The presence of soluble PD-1 (sPD-1) could act as competitors to the membrane bound PD-1, which, in turn, could limit the clinical benefit to anti-PD-1 antibodies. Similarly, the presence of soluble PD-L1 (sPD-L1) will limit the benefit of using anti-PD-L1 antibodies. On the other hand, harnessing immune checkpoint proteins other than PD-1/PD-L1 by tumor cell, such as cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and its ligand (B7) can limit the benefit to anti-PD-1/PD-L1 blockade therapy as seen in case (**c**). The absence of PD-1/PD-L1 axis will make the use of PD-1/PD-L1 blockade therapy useless as seen in case (**d**). As such, targeting both PD-1/PD-L1 and CTLA-4/B7 will optimize the therapeutic response in case (b). While targeting CTLA-4/B7 axis is supposed to be the right choice in case (**d**)

receptors (IFN-γRs) which, in turn, is important in promoting the expression of PD-L1 [49]. Therefore, reducing the expression of PD-L1 is associated with unresponsiveness or limited response to anti-PD1/PD-L1 immunotherapy in cancer patients. It is also important to mention that the benefit of immunotherapy increases in cancer patients as the level of expression of PD-L1 increases, some cancer patients still benefit from PD-1/PD-L1 blockade therapy even though their cancer cells do not express PD-L1 [36, 43, 50–53]. This is possibly because the expression of PD-L1 and CMTM6 is not solely limited to tumor cells, rather certain immune cells such as $CD68^+$ macrophages in the tumor stroma can also highly co-express PD-L1 and its positive regulator, CMTM6, as reported by Zugazagoitia et al. [54] These findings are consistent with the study of Mezzadra et al. [55] in that tumor-infiltrating immune cells such as DCs express both PD-L1 and CMTM6. However, Zugazagoitia et al. [54] noticed that the high level of expression of both PD-L1 and CMTM6 in tumor-infiltrating immune cells and stroma was associated with greater overall survival in treated patients. Hence, this could be implicated as a predictive strategy to determine the outcomes of immunotherapy, at least in certain cancer types, such as non-small cell lung cancer [54].

The overexpression of PD-1 on T cells and PD-L1 on tumor cells could also be considered as a strategy that limits the responsiveness to PD-1/PD-L1 axis inhibitors, especially, in the case where the ratio between anti-PD-1/PD-L1 antibodies and their cognate antigen targets is low, i.e., low titers of anti-PD-1/PD-L1 antibodies. As such, the lack or limited benefit of anti-PD-1/PD-L1 therapy in cancer patients can be attributed, at least in part, to the manipulation of PD-1/PD-L1 pathway by cancer cells to ensure resistance to immune responses. Therefore, studying the molecular pathways involved in PD-1/PD-L1 regulation is of central importance to pave the way to improve the anti-tumor immune responses in patients who are not undergoing anti-PD-1/PD-L1 therapy. This could also accelerate the development of new therapeutics that enhance the responsiveness to the currently available immune checkpoint inhibitors in cancer patients. To this end, we will now focus our discussion on the recently discovered positive regulators of PD-L1, namely CMTM6 and to a lesser extent CMTM4. Another important reason for focusing on this topic is the absence of a review that addresses the recent advances in this area. We will review the following: (1) A glance at CMTMs; (2) CMTM6 and to a lesser extent CMTM4 as positive regulators of PD-L1; (3) Other functions of CMTM6 in cancer biology; (4) Potential regulators of CMTM6 expression; (5) Shuttling CMTM6 through exosomes; (6) Blocking the interaction between PD-L1 and CMTM6; and (6) Soluble and intracellular PD-L1 and CMTM6.

Before we begin, it is important to remember that there are several mechanisms and pathways that positively regulate the expression of PD-L1. These include but are not limited to: (1) intrinsic genetic alterations in tumor cells such as amplification of the gene encoding PD-L1, namely 9p24.1 [56], and structural variations that lead to the disruption of the 3'-untranslated region of the *PD-L1* gene [57]; (2) post-transcriptional regulators such as certain microRNAs (e.g., miR-20b, miR-21, miR-130b); and (3) extrinsic factors that are not-genetically related such as those linked to hypoxia via hypoxia-inducible factor 1 (HIF1)-α [49], toll-like receptor (TLR)-4/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-activation pathway [58, 59], inflammatory cytokines (e.g., interleukin 'IL'-6, tumor-necrosis factor 'TNF'-α) that activate signal transducer and activator of transcription (STAT)-1, -2, and -3 pathways [49, 60, 61], and IFN-γ/IFN-γR activation pathways (e.g., IFNG, IRF1, IFN-types I and III), with the latter being a major regulator of PD-L1 [20, 55, 62–66], among others [67–70]. Again, in this review, we will focus only on the newly identified positive regulators of PD-L1, namely CMTM6 and CMTM4, according to the available data.

A glance at CMTMs

In recent years, it has become clear that the nine identified members of the chemokine-like factor (CKLF)-like MARVEL transmembrane domain-containing family (CMTMs; CKLF and CMTM1 to CMTM8) [71, 72] are widely expressed in different human cells/tissues and play a vital role in a variety of normal physiological events. These include but are not limited to the process of hematopoiesis (e.g., CMTM7 and CMTM8), immune response (e.g., CMTM2), vascular system development/function (e.g., CMTM3 and CMTM4), and fertility in males (e.g., CMTM1, CMTM2, CMTM3, and CMTM4), among others [73]. As such, abnormal expression and/or abnormal function of CMTMs have been reported, one way or another, in the pathogenesis of various pathological conditions, such as different types of cancer, autoimmune disorders, and infertility [73, 74]. At the molecular level, the genes encoding CKLF and CMTM members 1 to 4 are located on chromosome 16, while the gene encoding CMTM5 is located on chromosome 14, and those encoding the rest of the family members (CMTM6, CMTM7, and CMTM8) are located on chromosome 3. Structural investigations have shown that CKLF has at least four isoforms; two are produced as secreted isoforms (CKLF-1 and CKLF-3) and the other two (CKLF-2 and CKLF-4) as transmembrane isoforms. Functionally they act as chemokines, and also play a role in inflammation [71]. Therefore, it is not surprising that the members of CKLF exhibit a wide range of activities in humans. MARVEL is a unique domain consisting of four transmembrane-helices, and functionally with a close

relation/link to the membrane binding events, transport vesicles, as well as protein trafficking [66, 75–77].

CMTM6 and to a lesser extent CMTM4 as regulators of PD-L1

In 2017, Burr et al. [66] and Mezzadra et al. [55] were the first groups of investigators to identify CMTM6 as a master "positive" regulator for PD-L1. This is the first discovered function associated with this ubiquitously expressed type III transmembrane protein CMTM6. As previously mentioned, activation of the IFN-γ/IFN-γR pathway is known to be a major regulator pathway of PD-L1 expression. Interestingly, after screening more than 20,000 protein-coding genes in BxPC-3, a human pancreatic tumor cell line, using loss-of-function genetic screen technology by harnessing CRISPR-Cas9 system, Burr et al. [66] reported that the only protein-coding gene beside the already known major regulators of PD-L1 expression (namely, interferon-stimulated genes) was the *CMTM6* gene. Importantly, Burr et al. [66] reported that in the absence of IFN-γ stimulation, CMTM6 was the only regulator of PD-L1, but not PD-L2 which is the second ligand for PD-1. They also observed that stimulation with IFN-γ has no effect on the levels of CMTM6. Nevertheless, depletion of CMTM6 can significantly diminish the constitutive as well as the IFN-γ-induced expression of PD-L1 on the cell surface membrane. In addition, unlike other regulators of PD-L1 [78, 79], either in the presence or the absence of IFN-γ stimulation, CMTM6 did not seem to act as a regulator of PD-L1 at the transcription level. Instead, after performing reciprocal co-immunoprecipitation experiments under conditions that allow membrane solubilization to a variable degree, Burr et al. [66] indicated that, regardless of the presence or absence of IFN-γ stimulation, CMTM6 physically interacts and co-localizes with PD-L1 at the cell surface. Importantly, this event only occurs when the integrity of the membrane-associated complex is intact/preserved. In addition, the results of Burr et al. [66] indicated that CMTM6 is not involved in trafficking of PD-L1 from the endoplasmic reticulum (ER) to the surface of the plasma membrane, rather it plays vital role in stabilizing the expression of PD-L1 at the intact cell surface and in protecting PD-L1 from endosomal degradation during endosome recycling (Fig. 1).

Burr et al. [66] have demonstrated that targeting CMTM6 inhibition/depletion in cancer cells can enhance the activation of co-cultured cytotoxic ($CD8^+$) T cells (as measured by the increased proportion of TNF-α and perforin producing cytotoxic T-cells, as well as the increased IL-2 and IFN-γ production) and consequently render cancer cells susceptible to killing by cytotoxic T cells. It is worth mentioning that this effect is conditional, meaning that it will be beneficial in cancer settings where PD-1/PD-L1 is the major or the sole strategy by which cancer cells resist killing by T cells. Interestingly, CMTM6 does not affect the expression of major histocompatibility complex class I (MHC class I) while it shows a notable specificity for PD-L1. This feature, namely the neutral effect of CMTM6 on MHC class I expression, is important because one of the mechanisms that could limit the benefit from PD-1/PD-L1 blockade therapy in cancer patients is the impaired antigen(s)/neoantigen(s) presentation through MHC class I, which is in part due to the low or the lack/absence of MHC class I expression on tumor cells [80–85]. It is worth mentioning that MHC class I expression can be downregulated in cancer through different mechanisms including the targeting of MHC class I molecules for degradation through autophagy, activating proteasomal and lysosomal degradation pathways, or downregulating MHC class I gene expression, among others [82, 86–88] (Fig. 2). Burr et al. [66] compared the ability of mice to control tumor growth following transplantation with murine melanoma cancer cells expressing or lacking the expression of CMTM6. They found that the latter group had better survival and the results indicate that CMTM6 could be considered as a potential target for the treatment of cancer in the future.

At the same time, Mezzadra et al. [55] reported that CMTM6 is indeed a master regulator of PD-L1 in various cancer cell types including lung cancer, colorectal cancer, chronic myelogenous leukemia, thyroid cancer, and melanoma. Following analysis of 30 different types of cancer cells, a direct association between RNA levels of CMTM6 and PD-L1 was almost lacking for the majority of analyzed cancer cell types, indicating that CMTM6 is not involved in regulating the transcription of PD-L1. It is worth mentioning that treatment of haploid HAP1 cells, 8505C thyroid cancer cells, and A375 melanoma cells with IFN-γ is known to induce the expression of PD-L1 [55, 65, 89]. Interestingly, Mezzadra et al. [55] reported that depletion of CMTM6 resulted in 2-, 5-, and up to 11-fold reduction in the expression of PD-L1 on HAP1 cells, 8505C thyroid cancer cell line, and A375 melanoma cells, respectively, upon stimulation with IFN-γ. We must not forget that PD-L1 is not only expressed on tumor cells but also on tumor-infiltrating immune cells including dendritic cells (DCs). In view of that, Mezzadra et al. [55] generated DCs from human bone marrow progenitors and assessed the role of partial depletion of CMTM6 on the expression of PD-L1 in human bone marrow-generated DCs upon stimulation with lipopolysaccharide (LPS). Interestingly, a partial reduction in PD-L1 expression was observed on partially depleted CMTM6/LPS-stimulated bone marrow-generated DCs when compared to the control cells. They also reported that inhibition of CMTM6 in both cancer cell lines and DCs had an insignificant impact on inhibiting the expression of MHC class I and PD-L2 (also known as B7-DC or CD273). Mezzadra

et al. [55] also identified the stage(s) during the process of PD-L1 biosynthesis—from transcription to expression on the cell surface—that CMTM6 exerts its effect on PD-L1 biosynthesis. Importantly, they observed that the influence of CMTM6 on PD-L1 occurs when the PD-L1 protein leaves the ER. Their experiments also showed that PD-L1 always localizes in CMTM6 positive areas "primarily at the cell surface membrane where the CMTM6 is localized."

Additionally, Mezzadra et al. [55] reported that in the absence of CMTM6, ubiquitination of PD-L1 was a mechanism that drives the degradation of PD-L1, suggesting that CMTM6 may prevent the degradation of PD-L1 mediated by ubiquitination. Of note, in 2016, Lim and colleagues [90] reported that deubiquitination up-regulates the expression of PD-L1. To confirm this suggestion, Mezzadra et al. [55] assessed the role of STUB1, an E3 ubiquitin ligase that has been recognized as a negative regulator of PD-L1, on the expression of PD-L1 in cancer cells overexpressing or lacking the expression of CMTM6. Importantly, they found that disruption of STUB1 resulted in a more profound increase in expression of PD-L1 in cells that were deficient in CMTM6 when compared to cells that expressed CMTM6, indicating that STUB1 acts as a destabilizing "negative regulator" of PD-L1 (Fig. 1).

In fact, as the expression of PD-L1 increases, T cell survival and responsiveness decrease [57, 91]. Mezzadra et al. [55] investigated the impact of CMTM6 on T cell responses by co-culturing antigen-loaded cancer cells that either highly expressed or lacked the expression of CMTM6 with T cells expressing different levels of PD-1. Interleukin (IL)-2 was one of the measured analytes that was used to assess the responsiveness of T cells in this system. As expected, the production of IL-2 by T cells expressing intermediate and high levels of PD-1 upon encountering tumor cells expressing high levels of CMTM6 was profoundly decreased when compared to the tumor cells that lacked the expression of PD-1. However, the production of IL-2 from T cells was restored upon depletion of CMTM6 in tumor cells, indicating that the expression of CMTM6 influences T cells responses against tumor cells.

It is of particular importance to note that the remarkable variation in PD-L1 expression in different tumor cells in response to depletion of CMTM6. For example, in HAP1 cells there was a moderate effect (twofold reduction), whereas in A375 melanoma cells there was a profound reduction (up to 11-fold) in PD-L1 expression upon depletion of CMTM6. Consequently, Mezzadra et al. [55] proposed that other factor(s) could play a role in regulating PD-L1 expression, especially in cells that respond minimally to CMTM6 depletion such as HAP1 cells. Interestingly, Mezzadra et al. [55] identified CMTM4 (55% homology with CMTM6) as a back-up regulator of PD-L1 in the absence, but not in the presence, of CMTM6. However, CMTM4 is less effective than CMTM6 in upregulating PD L1. In agreement with these findings, several reports also confirmed the results of Burr et al. [66] and Mezzadra et al. [55] in that CMTM6 colocalizes with and stabilizes the expression of PD-L1 at the surface of tumor cells [54, 92–95]. Additionally, several groups of investigators including Mezzadra et al. [55], Zugazagoitia et al. [54] Zeisbrich et al. [95], Pang et al. [96], and Wu et al. [97] confirmed, the direct and/or indirect involvement of CMTM6 in regulating the expression of PD-L1 in different immune cells including DCs, macrophages, and monocytes.

Taken together, these results confirm that CMTM6 and, to a lesser extent, CMTM4 act as stabilizers of PD-L1 at the cell surface, namely at the protein level following biosynthesis, and are not involved in PD-L1 maturation. Targeting CMTM6/4 for inhibition does not affect the transcription of PD-L1 nor the trafficking of PD-L1 from the ER to the cell surface. Furthermore, it does not affect the expression of MHC class I. These findings suggest that targeting CMTM6 and/or CMTM4 proteins for inhibition could be of therapeutic value in cancer patients, especially, in those who are not undergoing PD-1/PD-L1 blockade therapy, as a strategy to enhance anti-tumor immunity by destabilizing the expression of PD-L1 on tumor cells, and thus minimizing/inhibiting its interaction with PD-1 on immune cells. Alternatively, promoting the expression of CMTM6 and/or CMTM4 could enhance the patient's response to PD-1/PD-L1 blockade therapy by enhancing the expression of PD-L1. This is, especially true, in patients who do not benefit at all or those who partially benefit from PD-1/PD-L1 blockade therapy, due to the lack of, or limited expression of, PD-L1 at the tumor cell surface [36, 50, 98]. In theory, the latter is rational only in the case where the lack of benefit is due to the lack of PD-L1 expression on tumor cells as a result of CMTM6-deficiency and/or -loss of function. However, it is important to mention that it has been recently discovered that unlike the expression of PD-L1, the expression of CMTM6 is a critical predictor of responsiveness to PD-1/PD-L1 blockade therapy, for example in non-small cell lung cancer (NSCLC) patients [99]. This is consistent with the previous observations in that the greater response to PD-1/PD-L1 blockade therapy in various cancer types, including NSCLC, could be predicted by different factors that are independent of the expression of PD-L1. Such factors include immune-related gene signatures [100], infiltration of cytotoxic T cells into the tumor-microenvironment [101], and high tumor mutational burden [102].

We must also remember that systemic inhibition/targeting of any protein "as a therapeutic strategy" will result in abnormal and toxic effects on normal cells/tissues, as is the case with the already used immune checkpoint inhibitors [103–107]. This is mainly due to lack of selectivity and specificity of the used therapeutic drugs to abnormal cells.

However, Burr et al. [66] and Mezzadra et al. [55] did not provide any information regarding the degree of cytotoxicity that could result from the systemic targeting of these proteins. Uncovering such information is important to evaluate CMTM6 and CMTM4 as potential targets for future therapeutics. The last important issue to be mentioned in this context is that Burr et al. [66] and Mezzadra et al. [55] did not investigate the mechanisms and factors that regulate the expression of CMTM6 in cancer cells, yet we extrapolated information from the available literature to gather the scattered results about this issue and we will discuss this later in this work.

Other functions of CMTM6 in cancer biology

Interestingly, besides the already discovered role played by CMTM6 in regulating the expression of PD-L1 on cancer cells and immune cells, new emerging roles in tumor biology have also been revealed recently [108–110]. For example, CMTM6 could be involved in tumorigenesis since downregulating the expression of CMTM6, using a specific short hairpin RNA (shRNA) for CMTM6, in different head and neck squamous cell carcinoma (HNSCC) cell lines significantly reduced their capacities to form colonies and renew themselves, and ultimately resulted in inhibition of cancer cell proliferation [108]. This indicates that high levels of CMTM6 are required for cancer cell proliferation. Chen et al. [108] also investigated the mechanisms by which CMTM6 could inhibit cancer cell proliferation. After analyzing most of the *CMTM6*-associated genes using the LinkedOmics database, *CTNNB1* (the gene encoding for β-catenin) showed the greatest correlation with *CMTM6*. These observations were also confirmed in HNSCC cell lines. The Wnt/β-catenin pathway is a well-known and highly conserved signaling pathway that plays various roles in the biology of cancer, such as tumor proliferation, epithelial-to-mesenchymal transition (EMT) and cancer stem cell-like phenotypes, of different cancer types (including HNSCC) [111–114]. Chen et al. [108] discovered a direct association between CMTM6 and β-catenin, since the tumor tissue regions that exhibited high levels of expression of CMTM6 were always abundant in β-catenin and vice versa. Importantly, knocking down the expression of CMTM6 resulted in a significant reduction in nuclear translocation, but not total expression, of β-catenin in HNSCC cell lines, indicating that CMTM6 is required for the nuclear translocation of β-catenin. Furthermore, CMTM6 was shown to play roles in acquisition of cancer stem cell-like properties and regulation of EMT in HNSCC cells. For the first time, the results of Chen et al. [108] confirmed that CMTM6 has other roles in cancer biology, and such results were also confirmed later by other investigators in other cancer cell types, including hepatocellular carcinoma and oral squamous cell carcinoma (OSCC) [93, 109, 110]. Furthermore, tumor recurrence in hepatocellular carcinoma has been linked to the increased expression of membrane CMTM6 in tumor cells [115]. A recent study has also indicated that CMTM6 drives cisplatin resistance via regulating Wnt signaling through the ENO-1/AKT/GSK3β axis [116]. Wang et al. [117] have also reported a positive correlation between the expression of CMTM6 and PD-L1 on lung adenocarcinoma (LUAD). Furthermore, their analysis showed a positive correlation between the expression of CMTM6 and immune cell infiltration to the LUAD tissues, suggesting the vital role played by CMTM6 in regulating immune cell infiltration in LUAD.

In another example, OSCC is among the most frequent cancers of the head and neck (most frequent oral cancers). In various solid tumors including OSCC, tumor-associated macrophages (TAM) with M2 (pro-tumor) phenotype (not M1 (anti-tumor) phenotype) play a critical role in the pathogenesis of cancer [118–120]. This is true since these macrophages have the capacity to shift the anti-tumor inflammatory microenvironment into a pro-tumor anti-inflammatory one through different mechanisms including, for example, the upregulation of PD-L1 expression [121–123] . Pang et al. [96] investigated the role of CMTM6 in this context and described several novel findings. Firstly, they reported that the expression of CMTM6 directly correlates with the infiltration of $CD163^+$ macrophages and PD-L1 expression and indirectly with the clinical characteristics in OSCC patients [96]. Knocking down the expression of CMTM6 in OSCC cell lines (Cal-27 and SCC25 cells) resulted in a significant reduction in cell proliferation, migration and invasion [96]. Moreover, the expression of PD-L1 was reduced upon silencing the expression of CMTM6 in Cal-27 and SCC25 cells. Additionally, CMTM6 was shown to be involved in polarization of macrophages to the M2 phenotype upon coculturing of non-polarized macrophages (M0) with OSCC cells using a Transwell system, suggesting that it could be involved in M2 polarization in OSCC microenvironment. Interestingly, according to the results of Pang et al. [96], polarization of macrophages into the M2 phenotype was induced in these coculture studies because OSCC cells shuttle CMTM6 to macrophages through exosomes. Moreover, Pang et al. [96] showed that activation of ERK1/2 signaling pathway was responsible for the polarization of M2 in M0/OSCC cells coculture experiments. Lastly, to confirm the in vitro results, Pang et al. [96] used a 4NQO-induced oral carcinoma animal model and downregulated the expression of CMTM6 using siRNAs specific to CMTM6. Interestingly, the in vivo results were consistent with the in vitro results in that downregulating the expression of CMTM6 resulted in a significant reduction in tumor progression, M2 polarization, and PD-L1 expression in treated mice when compared to the control mice. These results confirm that

CMTM6 plays a role in regulating the expression of PD-L1 in vitro and in vivo, and in the tumor progression of OSCC.

Two important messages stem from these findings: first, future investigations should not only focus on the role played by CMTM6 in regulating the expression of PD-L1 but should also extend to include other potential roles in cancer biology and other pathological conditions. Second, these findings are in full support with the view that the therapeutic value of targeting CMTM6 is increasing.

Regulators of CMTM6

Indeed, CMTM6 is becoming an important player in the pathology of various cancer types. Therefore, it is rational to conclude that targeting this protein will add a therapeutic value to the existing treatment regimens, at least in cancer settings. The absence of specific inhibitors for CMTM6 encouraged us to examine the literature to find more information regarding the existence of potential regulators for CMTM6. Fortunately, we have found that there are several potential regulators of CMTM6 including:

Circular RNAs (circRNAs)

CircRNAs are unique endogenous non-coding RNAs, and their roles in health and disease have become more apparent in recent years [124–127]. CircRNAs can modulate gene expression since they are involved in regulating transcription, as well as, protein and micro-RNA functions [127]. For example, circRNAs can bind to certain micro-RNAs (which are short stands of "18 to 22" nucleotides and play a critical role in regulating gene expression) that share complementary sequences with circRNAs. Thereby, circRNAs can act as endogenous anti-micro-RNAs or act as their competitors and consequently affect the expression of the micro-RNAs' targets [127, 128]. Cerebellar degeneration-related protein 1 transcript (CDR1-AS), is a circRNA, that is highly expressed, for example, in colorectal cancer, and is associated with poor prognosis [129]. This could be because CDR1-AS can target micro-RNA-7 [128, 130, 131]. In other words, the abundant expression of micro-RNA-7 in colorectal cancer is associated with good prognosis, especially, since it may have anti-tumor activity (tumor-suppressive activity) in the context of colorectal cancer [129, 131]. However, this is not the case when micro-RNA-7 is not expressed abundantly in colorectal cancer tissues, suggesting that CDR1-AS may have other functions in this context. Tanaka and colleagues [132] have reported that CDR1-AS upregulates the expression of CMTM6, possibly through manipulating the expression and/or function of transcription factors required for the expression of CMTM6, that in turn positively regulate the expression of PD-L1 on colorectal cancer. These findings may provide an explanation for the observed poor prognosis in colorectal cancer and/or other cancers that highly express CDR1-AS [131]. More importantly, these findings indicate that CDR1-AS acts as a positive regulator for the expression of CMTM6 and subsequently the expression of PD-L1 in cancer, suggesting that CDR1-AS could be considered as a therapeutic target. Yet, the mechanisms that regulate the high expression of CDR1-AS in certain cancers remain to be determined, and additional studies are required to further confirm the role of CDR1-AS as a positive regulator of CMTM6 on a larger scale.

WEE1 and ATM kinases

Facilitating DNA damage is considered to be a strategy to fight cancer cells [133]. WEE1 is a protein kinase located in the nucleus which becomes activated in response to DNA damage and regulates the response of the G2 checkpoint. It ultimately guides the cell to enter into a G2 phase arrest, thereby, preventing cell division via blocking entry to the mitotic phase [134]. WEE1 is among the factors that is known to be involved in the DNA repair system, i.e., it belongs to the cellular DNA damage response machinery, so once expressed/activated it enhances the survival of cells during cancer progression [135]. Ataxia telangiectasia mutated (ATM) kinase also belongs to the DNA damage response and shares certain goals with WEE1 kinase [135, 136]. Therefore, it is not surprising that these kinases are among the list of targets to combat cancers [135]. Jin et al. [133] investigated the impact of the experimental anti-cancer candidate inhibitors of WEE1 "AZD1775" and ATM "AZD0156" in pancreas cancer cell lines and in Capan-1 xenograft mouse model. They found that among the mechanisms by which these inhibitors exhibit anti-cancerous effect against pancreas cancer cells was by downregulation of the total cellular and surface PD-L1 on pancreatic cancer cells in vitro, especially in cancer cells that highly express PD-L1 such as SNU2913 cells, as well as in vivo [133]. Although this effect was observed when each inhibitor was used alone, the greatest effect was observed when the two inhibitors were applied in combination [133]. Importantly, the reduction in surface PD-L1 expression in these experiments was because of the reduction in the expression of CMTM6 and consequently reduction in binding of CMTM6 to PD-L1, which in turn could increase the degradation of PD-L1 through the lysosomal degradation pathway [133], as previously discussed.

It is worth mentioning that recent investigations have revealed that huntingtin interacting protein 1 related (HIP1R) protein, which is known to be involved in endocytosis and intracellular trafficking [137], directly interacts with PD-L1 and facilitates its delivery to lysosomes through

a lysosomal targeting signal and ultimately participates in the lysosomal degradation of PD-L1 [138]. Although both HIP1R and CMTM6 can directly "physically" interact with PD-L1, the function of HIP1R counteracts the function of CMTM6. This may indicate that HIP1R and CMTM6 might be considered as competitors in this context. As such, Jin et al. [133] assumed that the reduction in the expression of PD-L1 by WEE1 and ATM inhibitors could be because they reduced the expression of CMTM6 or increased the expression of HIP1R [133], or both. Taken together, these findings suggest that WEE1 and ATM enzymes are involved in regulating the expression of CMTM6 and its interaction with PD-L1. However, these assumptions need to be confirmed and the exact mechanisms by which such effects are driven remain to be determined in future investigations.

Hu-antigen R (HuR)

HuR is an RNA-binding protein that belongs to the embryonic lethal abnormal vision-like (ELAV) protein 1 family, which contains the four members HuR, HuB, HuC, and HuD. HuR has been reported to be overexpressed in various cancer types, and to play critical roles in promoting tumor progression [139–145]. This is mainly because of the biological functions of HuR, that include critical roles in mRNA splicing, stabilization (which can be achieved in part through binding to AU-rich elements "AREs" to antagonize degradation signals) and translation [142, 143, 146]. In the context of regulating CMTM6, a remarkable recent study has revealed the direct involvement of HuR in regulating the mRNA of CMTM6 in various human cancers [147]. In contrast to the cancer cells lines ACHN and 769-p cells with downregulated HuR expression, cancer cell lines 786–0 and Caki-1 cells that highly express HuR were shown to express high levels of CMTM6. HuR was able to regulate the mRNA of CMTM6 through binding to certain AREs motifs in 3'UTR of CMTM6 mRNA. Initially, these results indicate that HuR is a direct positive regulator of CMTM6 at the transcript level. Overexpression of HuR resulted in a remarkable increase in PD-L1 expression upon stimulation with IFN-γ and vice versa, and regardless of the expression level of HuR, transcription and translation of PD-L1 were not affected. The predominant role played by CMTM6 in HuR-upregulated PD-L1 expression was also confirmed, since downregulating the expression of CMTM6 in cells that were overexpressing HuR attenuated the PD-L1 expression induced by IFN-γ. At the same time, the expression of PD-L1 induced by IFN-γ in cells with low HuR expression was augmented upon complementation with CMTM6. These results indicate that the upregulation of PD-L1 by HuR was due to its ability to stabilize the transcripts of CMTM6. As such, these results have revealed a new mechanism (i.e., inhibition of immune responses through CMTM6/PD-L1) by which HuR contributes to the pathogenesis of tumor development beside the already discovered mechanisms [142, 148, 149]. Co-culture experiments showed that high expression levels of HuR in cancer cells significantly inhibited immune response (i.e., production of IL-2 by T cells), and such events were completely abolished upon treatment with a specific inhibitor of HuR. In the absence of CMTM6 inhibitors, HuR could be considered as an alternative target, especially because of the presence of cell permeable-specific HuR inhibitors (such as MS-444, which can bind to HuR and perturb the interaction between HuR and the AREs of different mRNA targets [150]).

Epithelial-to-mesenchymal transition (EMT)

EMT is a physiological process whereby epithelial cells switch their phenotype into mesenchymal phenotype, which is known to be associated with a more motile and invasive phenotype, that, in turn, are essential for embryogenesis and wound healing, and in malignancy they can be exploited to promote tumor progression [151, 152]. EMT is regulated by different transcription factors that belong to the TWIST, ZEB, and SNAIL (also known as SNAI1) families [152, 153]. It is worth mentioning that, for example, ZEB1 and SNAIL can bind to the proximal promoter of PD-L1 and induce its expression in lung and breast cancer cells [67, 154], and as such EMT is considered as a strategy to increase the expression of PD-L1. While studying the role of EMT-transcription factors in regulating immune checkpoints after the translation step, Xiao et al. [151] observed that induction of EMT by SNAIL increased the expression of CMTM6 in mesenchymal breast cancer cells. Silencing the expression of CMTM6 by a specific siRNA in MDA-MB-231 cells strongly decreased the expression of PD-L1 on these cells. These findings indicate that EMT transcription factors, particularly SNAIL, play a role in upregulating the expression of CMTM6. Of note, an additional novel finding in this study was that the overexpression of PD-L1 on mesenchymal tumor cells is guided by the co-expression of two members of the CMTM family, namely CMTM6 and CMTM7, since the inhibition of CMTM6 and CMTM7 together showed a synergistic effect in reducing the expression of PD-L1 on cancer cells.

Certain therapeutics

It has recently been observed that certain therapeutics could play a role in increasing the expression of CMTM6. One example is the effect of the anti-hepatitis B virus medications Entecavir and Lamivudine on the expression of PD-L1. These commonly used medications inhibit HBV replication through blocking the viral reverse transcriptase activity. Yamamoto and colleagues [155], observed that treatment of

HBV infected hepatocytes by Lamivudine increases the levels of PD-L1 at the mRNA transcription level with no effect on cell surface expression. In contrast, Entecavir increased the cell surface expression of PD-L1 on HBV infected hepatocytes in a dose-dependent manner, in part, through upregulating the expression of CMTM6. However, the mechanisms by which Entecavir increases the expression of CMTM6 are still unknown, requiring additional investigations.

All in all, although these findings are important because they open new insights in this research area, it seems that our knowledge about the regulatory processes involved in CMTM6 expression is still in its infancy and thus additional investigations are needed.

Shuttling CMTM6 through exosomes increases the expression of PD-L1 in tumor stroma

It has been recently discovered that tumor cells may shuttle CMTM6 through exosomes to the surrounding cells to increase the expression of PD-L1 on tumor stroma cells, including immune cells (Fig. 1) [96]. This, in turn, contributes to the enhancement of tumor progression by suppressing anti-tumor immune responses through converting the tumor microenvironment into an immune-suppressive microenvironment and/or by limiting the response to certain anticancer therapeutics as previously mentioned. These findings have extended our understanding about the role of CMTM6 in the pathology of cancer. Accordingly, targeting the expression of CMTM6 may provide a real therapeutic target for cancer.

Blocking the PD-L1/CMTM6 interaction as a therapeutic strategy

Investigations have shown that blocking the interaction of PD-L1 with CMTM6 by the specific monoclonal antibody, H1A (which targets the epitope 20–32 aa), but not the FDA approved anti-PD-L1 antibodies (durvalumab and atezolizumab), can destabilize the expression of PD-L1 and enhance the lysosomal degradation of PD-L1 upon endosomal recycling [30] (Fig. 1), both of which result in reduced expression on the surface of target cells. This strategy is considered as a therapeutic strategy to decrease the expression of PD-L1.

It is important to remember that tumor cells are not the only cells to express PD-L1 on their surfaces, since certain tumor-reactive T cells (tumor-infiltrating T lymphocytes; TIL) are also known to express PD-L1 [35] and thus will also serve as targets for PD-1/PD-L1 blockade therapy, apart from the intended PD-L1 expressing tumor cells. Accordingly, the response to PD-1/PD-L1 blockade therapy may also be affected by the level of tumor-reactive T cells expressing PD-L1 [35, 156]. Importantly, some investigations have shown that not all anti-PD-L1 antibodies have the same effect on these types of T cells, resulting in different T cell responses that could lead to different outcomes at the clinical level [157, 158]. In part, this could be due to the different mode of interaction of anti-PD-L1 antibodies with PD-L1, i.e., different binding sites but not the isotype of the used antibodies, which consequently would activate different signaling pathways, according to the epitope binding site [159, 160]. For example, the loss of anti-tumor activity of $CD8^+$ T cells and induction of T cell apoptosis were observed upon exposure to certain anti-PD-L1 antibodies, such as H1A monoclonal antibody which activates p38 MAPK through association with DNA-dependent protein kinase [159]. Therefore, such antibodies could have a greater therapeutic potential than the already existing antibodies against immune checkpoint proteins.

Soluble and intracellular PD-L1 and CMTM6

Another important issue to be taken into consideration in this regard is that recent investigations have revealed that beside the existence of PD-1 and PD-L1 on the surface membrane of cells as a membrane-bound protein, these immune checkpoint proteins also exist in an extracellular (soluble PD-1 "sPD-1" and soluble PD-L1 "sPD-L1") form [161]. In different cancer types, the increased expression of sPD-L1 has been regarded as a negative prognostic factor [162]. In various cancer types, the increased levels of sPD-1 post-treatment, but not pre-treatment, is associated with improved survival. This may be because sPD-1 could bind to both the cellular and the extracellular PD-L1, thereby acting as anti-PD-L1. In case of treatment with anti-PD-1 antibodies, sPD-L1 could act as a competitor to the membrane-expressed PD-L1 on tumor cells, thereby, as the expression level of sPD-L1 increases the benefit of anti-PD-L1 antibody therapeutics may be affected (Fig. 2). However, the role of CMTM6 expression on the expression of sPD-L1 has not been investigated, and thus, future investigations are required to fill the gap of knowledge in this context.

Investigations have also been extended to explore not only the extracellular but also the intracellular "non-immunological" role(s) played by PD-L1. Interestingly, it has been discovered, for the first time, that intracellular PD-L1 can positively regulate different DNA damage related genes such as Nijmegen breakage syndrome 1 (*NBS1*) and breast cancer 1 (*BRCA1*) genes, among others, by regulating the stability of mRNAs of these genes, since the intracellular PD-L1 has been shown to behave like an RNA binding protein [30].

This function was shown to be completely independent of the extracellular interaction of PD-1 and PD-L1. Therefore, intracellular PD-L1 enhances cellular resistance to DNA damage as it protects targeted RNAs from degradation by RNA exosomes. This was confirmed upon assessing the impact of PD-L1 knockdown both in cells that are highly expressing PD-L1 (e.g., HCT116 and MDA-MB-231 cell lines) and also in cells that almost lack expression (e.g., HeLa and A549 cells lines) with regard to sensitivity to chemotherapy (cisplatin) and to ionizing radiation. Importantly, knocking down the expression of PD-L1 resulted in a significant increase in sensitivity of cancer cells to both cisplatin and ionizing radiation [30]. Restoring the expression of PD-L1 reversed the sensitivity of cancer cells to cisplatin and ionizing radiation [30]. Of note, the role of CMTM6 and/or other CMTMs in the expression of the intracellular PD-L1 has yet to be investigated.

Conclusion

The expression of PD-L1 (a potent immunosuppressor) in several cancer types has been associated with unwanted clinical outcomes. PD-L1 expression also increases tumor resistance to certain anti-tumor therapies. Furthermore, the limited response to anti-PD1/PD-L1 therapeutics in clinical cancer settings, has encouraged investigators to determine the mechanisms and factors that regulate the expression of PD-L1. Fortunately, recent investigations have identified CMTM6 and to a lesser extent CMTM4 as stabilizers of PD-L1 protein at the cell surface following its biosynthesis (meaning that they are not involved in PD-L1 maturation). Initial investigations have revealed that targeted inhibition of CMTM6/4 does not affect the transcription of PD-L1 nor its trafficking from the ER to the cell surface. In addition, it does not affect the expression of MHC class I. Beside the newly identified roles played by CMTM6 in tumor biology (e.g., EMT, invasion, metastasis among others), these findings collectively suggest that targeting CMTM6 and/or CMTM4 proteins for inhibition could be of therapeutic value in cancer patients. This is especially the case for patients that are not undergoing PD-1/PD-L1 blockade therapy, as a strategy to enhance anti-tumor immunity by destabilizing the expression of PD-L1 on tumor cells, and thus minimizing/inhibiting its interaction with PD-1 on immune cells and consequently activating anti-tumor immune responses. Alternatively, targeting CMTM6 and/or CMTM4 expression could enhance the responses to PD-1/PD-L1 blockade therapy by enhancing the expression of PD-L1, especially, in patients who do not benefit or who partially benefit from PD-1/PD-L1 blockade therapy due to the lack of or limited expression of PD-L1 at the surface of tumor cells. In theory, the latter is rational only in the case where the lack of benefit is due to the absence of PD-L1 expression on tumor cells as a result of CMTM6-deficiency and/or CMTM6-loss of function. However, it is important to mention that it has recently been reported that unlike PD-L1, the expression of CMTM6 was a critical predictor of responsiveness to PD-1/PD-L1 blockade therapy in certain cancer types, for example in non-small cell lung cancer (NSCLC) patients. This is consistent with previous observations in that the greater response to PD-1/PD-L1 blockade therapy in various cancer types including NSCLC could be predicted by different factors that are independent of the expression of PD-L1, such as immune-related gene signatures, infiltration of cytotoxic T cells into the tumor-microenvironment, and high mutational burden of the tumor.

To determine the mechanisms and factors that regulate the expression of CMTM6 we extrapolated information from the available literature and surprisingly found that circRNAs, WEE1 and ATM kinases, HuR protein, and certain EMT related transcription factors such as SNAIL, are involved, in some way, in regulating the expression of CMTM6. Therefore, targeting these factors may provide an alternative approach for targeting CMTM6 in the absence of specific CMTM6 inhibitors. Although these findings are important in terms of gaining new insights in this research area, it seems that our knowledge about the processes involved in regulation of CMTM6 is still in its infancy and therefore additional investigations are needed. Alternatively, blocking the interaction of CMTM6 with PD-L1 could drive the degradation of PD-L1, and consequently result in reducing its expression.

With respect to the role of CMTM6 in regulating the expression of sPD-L1 there is no available information, and as such, we encourage investigators to open this door, especially because of the emerging role(s) of the soluble form of PD-L1 in cancer biology, as well as, its involvement in response to treatments in cancer patients.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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