Review

4-1BB and the Epigenetic Regulations of This Molecule

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Key Words

4-1BB · 4-1BBL · Epigenetic regulation · Histone acetylation

Abstract

4-1BB, a master regulator of our defense system, is present on several kinds of immune cells and has different functions in immune responses based on specific conditions. An expression of this molecule on T lymphocytes, antigen-presenting cells (APCs) and pathogenic cells directs immune responses by a costimulatory signal of *4-1BB* and its ligand, *4-1BBL*. Under abnormal conditions, such as inflammation and hypoxia, *4-1BB* and *4-1BBL* are also induced on nonimmune cells including epithelial cells, endothelial cells, smooth muscle cells, and cardiac myocytes. Recently, *4-1BB* has been found on brite adipocytes; it is identified as a specific marker for this type of fat cells. An increase in acetylated histone by histone deacetylase inhibitors (HDACi) leads to an elevation of *4-1BB* and *4-1BBL* expression and major histocompatibility complex expression on T-cell lymphoma and other tumor cell lines, which enhance the activities of APCs and cytotoxic T lymphocytes to improve antitumor immune responses. Conversely, *4-1BB* signaling triggered by a soluble 4-1BB receptor or anti-4-1BB antibodies strengthens the anticancer effect of HDACi by regulating both effector and regulatory T cells. Therefore, further investigations into the epigenetic regulations of *4-1BB/4-1BBL* interaction will give us more meaningful information to develop new methods to prevent disorders in human beings such as cancer, obesity, autoimmune and infectious diseases.

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Introduction

In the tumor necrosis factor (TNF) receptor family, *4-1BB* has emerged as a key member in controlling the survival and proliferation of immune cells, especially CD8⁺ lymphocytes [1]. *4-1BB* and *4-1BBL* are selectively present on activated cells of defense systems and on some nonimmune cells under identified conditions, such as the inducible expression of *4-1BB* on

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activated T cells, dendritic cells (DCs) or tumor cells in cancer [2–7]. Due to the selective expression of *4-1BB* and *4-1BBL* on specific cells under certain conditions, 4-1BB-expressing cells are considered as targets of treatment in pathology [3] and as targets for the development of new methods to deliver a specific antigen or doxorubicin to treat a disease [8].

The discoveries of *4-1BB* and *4-1BBL* are applied in preclinical trials such as the administration of agonistic and antagonistic 4-1BB or 4-1BBL monoclonal antibodies to prevent cancer, autoimmunity [3, 9–14] and infectious diseases [15]. *4-1BB*, *4-1BBL* and these antibodies are used to enhance the defense system of the body against pathogens [16] and to improve the vaccine effect [17–19]. The combination of 4-1BB antibodies with other reagents and methods to prevent diseases brings some positive results, such as the combination with IL-12 or histone deacetylase inhibitors (HDACi) in tumor treatment [20, 21].

Epigenetic modifications regulate several elements of the immune system. The variation of genomic DNA methylation among cytokine genes and T cells impacts on cytokine expression in primary T cells, such as interferon- γ (IFN- γ) and interleukin 3 (IL-3) in CD8⁺ T lymphocytes [22]. An increase in histone acetylation at the IFN- γ and IL-4 loci is accompanied by Th1/Th2 differentiation, which is maintained by transcription factors, *Tbet* and *GATA3*, in a STAT-dependent manner [23].

FoxP3, a master regulation transcription factor of regulatory T cells (Treg cells), is directed by the status of DNA methylation. Methylated DNA of *FoxP3* is significantly associated with Treg dysfunction and the upregulation of IgE during polycyclic aromatic hydrocarbon exposure [24] in atopic children. However, DNA methylation of a cyclic AMP response element binding protein/activating transcription factor inversely correlates with *FoxP3* expression [25]. The interaction of *Fas* (*CD95/TNFRSF6*) and its ligand (*FasL/CD95L/TNFSF6*) initiates apoptosis in lymphoid and nonlymphoid tissues, and this signal is controlled in an epigenetic manner. Chromatin remodeling modulates the *Fas* expression in primary leukemia T cells, but mechanisms underlying the repression of *Fas* expression are independent of protein deacetylation and DNA methylation of the *Fas* promoter region [26].

TNF Receptor Superfamily, Member 9 (4-1BB)

4-1BB (*CD137*, *TNFRSF9*) is a member of the TNF receptor family. It is popularly known as an inducible costimulatory molecule expressed on activated T lymphocytes such as CD4⁺, CD8⁺ and natural killer T cells (fig. 1) [2–5]. Activated natural killer cells, DCs, eosinophils, and mast cells also express this molecule, but myeloid-derived suppressor cells, a heterogeneous population of immune cells from the myeloid lineage, fail to express surface 4-1BB [6]. Although this marker is normally induced on stimulated immune cells, FoxP3⁺ regulatory CD4⁺ T cells express constitutively high levels of *4-1BB* [7]. Furthermore, under abnormal conditions, 4-1BB is present on nonhematopoietic cells including endothelial cells, smooth muscle cells and cardiac myocytes (fig. 1) [7].

The ligand of *4-1BB* (*4-1BBL/TNFSF9/CD137L*) is found on activated macrophages, DCs and B cells, and it is also expressed on some nonhematopoietic cells including endothelial cells, fibroblasts and epithelial cells [7]. *4-1BB* and its ligand are considered as attractive targets for modulating immune responses in vivo because it is expressed on activated, but not resting, T cells, and the ligation of this molecule by either *4-1BBL* or agonistic antibodies provides a potent costimulatory signal [13]. The 4-1BB signal in T lymphocytes and antigen-presenting cells (APCs) increases cellular proliferation, cytokine production and survival, so *4-1BB* regulates both effector and Treg cells [1]. Thus, immune modulation of *4-1BB* triggered by recombinant proteins and anti-4-1BB agonist antibodies is a promising therapy to control inflammation, infectious and cancer immunity as well as autoimmunity.

Fig. 1. Cells and diseases are influenced by 4-1BB signaling. 4-1BB is selectively expressed on immune or nonimmune cells under specific conditions. Therefore, 4-1BB signaling targets on identified cells and health conditions.

The interaction of 4-1BB and its ligand plays complex roles in regulating immune responses under basal or disease conditions [7, 27]. A costimulatory signal of this ligation inhibits apoptosis by upregulating antiapoptotic molecules such as *Bcl2* and *Bcl-xl* and protects tumor antigen-specific cells from activation-induced cell death [28]. During alloimmune responses, both alloreactive T cells, CD8⁺ and CD4⁺ T lymphocytes, express 4-1BB, despite their difference in the patterns of 4-1BB expression [10]. An active form of autoreactive CD4⁺ T cells with the expression of 4-1BB modulates autoimmune diseases by activating macrophages, inducing proinflammatory cytokines and helping B cells to produce autoantibodies [3, 5, 29]. An administration of agonistic anti-4-1BB monoclonal antibodies effectively eliminates certain autoimmune diseases such as autoimmune encephalitis, lupus-like autoimmunity, rheumatoid arthritis and chronic graft-versus-host disease [3, 11, 12, 29, 30].

Surprisingly, nowadays, 4-1BB is regarded as one of the specific signatures of brite adipocytes, the latest and third type of fat cells [31]. These adipocytes are induced by *Pparγ* agonists in vitro from preadipocytes of white fat depots [32, 33] and by cold or β-adrenergic receptor agonists in vivo in white fat tissues as subcutaneous and visceral white fat tissues [34–36]. Importantly, brite adipocytes are present in both infant and adult humans [31, 37, 38]; with thermogenic functions like those of the classical brown adipocytes, they are potential goals for developing new methods to prevent obesity and its related disorders in human beings.

Epigenetic Regulations of 4-1BB

It seems that most epigenetic studies on 4-1BB and its ligand are investigations into the effect of HDACi on these molecules in cancer immunity [39–41]. HDACi such as SAHA (suberoylanilide hydroxamic acid)/vorinostat, trichostatin A and MS-275 induce apoptosis of leukemic blasts by activating the death receptor pathway and transcriptional induction of the TNF-related proapoptotic family members, *TRAIL* and *FasL* [39]. HDACi treatment increases the expression and activity of 4-1BBL in leukemia cell lines, but this action of HCACi does not need de novo protein synthesis and DNase I hypersensitive chromatin remodeling [39]. A class I HDACi, MGCD0103, increases the expression of 4-1BB, *TNFSF4* (*OX40L*) and *TNF*, genes

involved in inflammation, and upregulates the expression of genes regulating *IFN- γ* , *IL-6*, *IL-8* and *IL-23* signaling pathways in the human Hodgkin cell lines [40]. Furthermore, proliferation and *TFN- γ* secretion of T lymphocytes are upregulated by MS-275, but this effect is reduced by a 4-1BB-blocking recombinant protein [39]. In colorectal carcinoma cell lines, inhibition of histone deacetylases (using trichostatin A) and DNA methyltransferases (using 5-aza-2'-deoxycytidine) and radiation increase the expression of *OX40L* and *4-1BBL* at both protein and mRNA levels [42]. These results indicate that histone acetylation enhances the expression and function of *4-1BB* and *4-1BBL* in leukemia cells; conversely, 4-1BB signaling is required for epigenetic regulations in tumor immunity.

In a model of human breast cancer, a combined treatment of SAHA (vorinostat), a HDACi, with a soluble 4-1BB receptor induces an upregulation of *4-1BB* and *4-1BBL* with a synergistic cytotoxic effect [21]. Furthermore, anti-4-1BB plus anti-CD40 antibodies enhance the anti-lymphoma effect of HDACi in mouse models [41]. The combined treatment of immune-activating antibodies and HDACi increases the function of APCs and elevates the proliferation and survival of cytotoxic T cells of the immune system, resulting in the eradication of established solid tumors [41]. Whereas irradiation upregulates histone H3 acetylation of *4-1BBL* promoters in tumor cell lines, coculture of T cells with radiation or trichostatin-A-treated tumor cells enhances the survival and activation of T cells [42]. These findings indicate that 4-1BB/4-1BBL signaling enhances the anticancer effect of HDACi and radiation.

In vitro, RIP140 suppresses the expression of *4-1BB* in brite adipocytes developed from subcutaneous white adipose tissues of mice [43]. It also silences the expression of *Ucp1*, a specific marker of brite and brown adipogenesis, in cultured adipocytes derived from mouse embryonic fibroblasts by modulating histone acetylation and DNA methylation [44]. This result seems to indicate that RIP140 reduces *4-1BB* expression and brite adipogenesis in white fat tissues through an epigenetic dependent pathway.

Conclusions and Future Directions

Accumulating evidence shows that *4-1BB* is a powerful regulator of immune responses by modulating survival, antigen presentation, activation and activity of immune cells (fig. 1). The expression of this marker on tumor cells enhances antitumor immunity by increasing the proliferation and activity of APCs and effector T cells. The expression of *4-1BB* on pathogenic cells and immune cells is at least affected by the acetylation status of histones. The increase in the anticancer effect by a combined treatment of anti 4-1BB antibodies and HDACi encourages us to investigate more epigenetic regulations of 4-1BB in cancer and other disorders in human beings such as obesity, autoimmune and infectious diseases.

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