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.

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\textsuperscript{+} 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
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 4-1B 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.
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, MGC0103, increases the expression of 4-1BB, TNFSF4 (OX40L) and TNF, genes

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**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.
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 TNF-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 antilymphoma 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.

References

1 Wang C, Lin GHY, McPherson AJ, Watts TH: Immune regulation by 4-1BB and 4-1BBL: complexities and challenges. Immuno Rev 2009;229:192–215.
2 Kwon BS, Weissman SM: cDNA sequences of two inducible T-cell genes. Proc Natl Acad Sci USA 1989;86:1963–1967.
3 Vinay D, Kwon B: Immunotherapy targeting 4-1BB and its ligand. Int J Hematol 2006;83:23–28.
4 Pollok K, Kim Y, Zhou Z, Hurtado J, Kim K, Pickard R, Kwon B: Inducible T cell antigen 4-1BB. Analysis of expression and function. J Immunol 1993;150:771–781.
5 Croft M: The role of TNF superfamily members in T-cell function and diseases. Nat Rev Immunol 2009;9:271–285.
6 Dubrot J, Aziplikuetu A, Alfaro C, Murillo O, Arina A, Berraondo P, Hervás-Stubbis S, Melero I: Absence of surface expression of CD137 (4-1BB) on myeloid-derived suppressor cells. Immunology 2007;126:121–126.
7 Kwon B: CD137-CD137 ligand interactions in inflammation. Immune Netw 2009;9:84–89.
25 Kim H-P, Leonard WJ: CREB/ATF-dependent T cell receptor-induced autophagy. Cancer Res 2010;70:3945–3954.

9 Kocak E, Lute K, Chang X, May KF, Exten KR, Zhang H, Abdessalam SF, Lehman AM, Jarjoura D, Zheng P, Liu Y: Combination therapy with anti-CTLA-4 and anti-4-1BB antibodies enhances cancer immunity and reduces autoimmunity. Cancer Res 2006;66:7276–7284.

10 Cho HR, Kwon B, Yagita H, La S, Lee EA, Kim JE, Akiba H, Kim J, Suh JH, Vinay DS, Ju SA, Kim BS, Mittler RS, Okumura K, Kwon BS: Blockade of 4-1BB (CD137)/4-1BB ligand interactions increases allograft survival. Transpl Int 2004;17:351–361.

11 Kim J, Choi WS, La S, Suh J-H, Kim BS, Cho HR, Kwon BS, Kwon BS: B-cell Stimulation with 4-1BB (CD137) inhibits chronic graft-versus-host disease by inducing activation-induced cell death of donor CD4+ T cells. Blood 2005;105:2206–2213.

12 Seo SK, Choi JH, Kim YH, Kang WJ, Park HY, Suh JH, Choi BK, Vinay DS, Kwon BS: 4-1BB-mediated immunotherapy of rheumatoid arthritis. Nat Med 2004;10:1088–1094.

13 Martin-Orozco N, Dong C: Inhibitory costimulation and anti-tumor immunity. Semin Cancer Biol 2007;17:288–298.

15 Lee S-C, Ju S-A, Sung B-H, Heo S-K, Cho HR, Lee EA, Kim JD, Lee IH, Park S-M, Nguyen Q-T, Suh J-H, Kim B-S: 4-1BB stimulation enhances host defense of mice against *Listeria monocytogenes* infection by inducing rapid infiltration and activation of neutrophils and monocytes. Infect Immun 2009;77:2168–2176.

16 Shin SM, Kim YH, Choi BK, Kwon PM, Lee H-W, Kwon BS: 4-1BB triggers IL-13 production from T cells to limit the polarized, Th1-mediated inflammation. J Leukoc Biol 2007;81:1455–1465.

17 Munks MW, Mourich DV, Mittler RS, Weinberg AD, Hill AB: 4-1BB and OX40 stimulation enhance CD8 and CD4 T-cell responses to a DNA prime, poxvirus prime vaccine. Immunology 2004;112:559–566.

18 Kudo-Saito C, Hodge JW, Kwak H, Kim-Schulze S, Schilom J, Kaufman HL: 4-1BB ligand enhances tumor-specific immunity of poxvirus vaccines. Vaccine 2006;24:4975–4986.

19 Calarota SA, Hokey DA, Dai A, Jure-Kunkel MN, Balimane P, Weiner DB: Augmentation of SIV DNA vaccine-induced cellular immunity by targeting the 4-1BB costimulatory molecule. Vaccine 2008;26:3121–3134.

20 Pan P-Y, Gu P, Li Q, Xu D, Weber K, Chen S-H: Regulation of dendritic cell function by NK cells: mechanisms underlying the synergism in the combination therapy of IL-12 and 4-1BB activation. J Immunol 2004;172:4779–4789.

21 Bellarosa D, Bressan A, Bigioni M, Pariani M, Maggi C, Binasci M: SAHA/Vorinostat induces the expression of the CD137 receptor/ligand system and enhances apoptosis mediated by soluble CD137 receptor in a human breast cancer cell line. Int J Oncol 2012;41:8.

22 Fitzpatrick DR, Shirley KM, McDonald LE, Bielefeldt-Ohmann H, Kay GF, Kelso A: Distinct methylation of the interferon γ (IFN-γ) and interleukin 3 (IL-3) genes in newly activated primary CD8+ T lymphocytes: regional IFN-γ promoter demethylation and mRNA expression are heritable in CD44highCD8+ T cells. J Exp Med 1998;188:103–117.

23 Fields PE, Kim ST, Flavell RA: Cutting edge: changes in histone acetylation at the IL-4 and IFN-γ loci accompany Th1/Th2 differentiation. J Immunol 2002;169:647–650.

24 Hew KM, Walker AJ, Kohli A, Garcia M, Syed A, McDonald-Hymann C, Noth EM, Mann JK, Pratt B, Balnes J, Hammond SK, Eisen EA, Nadeau KC: Childhood exposure to ambient polycyclic aromatic hydrocarbons is linked to epigenetic modifications and impaired systemic immunity in T cells. Clin Exp Allergy 2014, Epub ahead of print.

25 Kim H-P, Leonard WJ: CREB/ATF-dependent T cell receptor-induced Foxp3 gene expression: a role for DNA methylation. J Exp Med 2007;204:1543–1551.

26 Castellano R, Vire B, Pion M, Quivy V, Olive D, Hirsch I, Van Lint C, Collette Y: Active transcription of the human FASL/CD95L/TNFSF6 promoter region in T lymphocytes involves chromatin remodeling: role of DNA methylation and protein acetylation suggest distinct mechanisms of transcriptional repression. J Biol Chem 2006;281:14719–14728.

27 Park S, Kim H, Lee J, Cho H, Kwon B: Reverse signaling through the co-stimulatory ligand, CD137L, as a critical mediator of sterile inflammation. Mol Cells 2012;33:533–537.

28 Moran AE, Kociosovcs-Bankowski M, Weinberg AD: The TNFRs OX40, 4-1BB, and CD40 as targets for cancer immunotherapy. Curr Opin Immunol 2013;25:230–237.

29 Mittler R, Foell J, McCausland M, Strahotin S, Niu L, Bapat A, Hewes L: Anti-CD137 antibodies in the treatment of autoimmune disease and cancer. Immunol Res 2004;29:197–208.

30 Foell J, Strahotin S, O’Neill SP, McCausland MM, Haber M, Chander PN, Bapat AS, Yan X-J, Chiorazzi N, Hoffman MK, Mittler RS: CD137 costimulatory T cell receptor engagement reverses acute disease in lupus-prone NZB × NZW F1 mice. J Clin Invest 2003;111:1505–1518.

31 Wu J, Boström P, Sparres LM, Ye L, Choi JH, Giang A-H, Khandekar M, Virtanen KA, Schrauwen P, Spiegelman BM: Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 2012;150:366–376.
Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedegaard J: Chronic peroxisome proliferator-activated receptor γ (PPARγ) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. J Biol Chem 2010; 285: 7153–7164.

Chu D-T, Malinowska E, Gawronska-Kozak B, Kozak LP: Expression of adipocyte biomarkers in a primary cell culture model reflects preweaning adipobiology. J Biol Chem 2014; 289: 18478–18488.

Lončar D, Afzelius BA, Cannon B: Epididymal white adipose tissue after cold stress in rats. I. Nonmitochondrial changes. J Ultrastruct Mol Struct Res 1988; 101: 109–122.

Guerra C, Koza RA, Yamashita H, Walsh K, Kozak LP: Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. J Clin Invest 1998; 102: 412–420.

Himms-Hagen J, Cui J, Danforth E Jr, Taatjes DJ, Lang SS, Waters BL, Claus TH: Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. Am J Physiol 1994; 266:R1371–R1382.

Cypess AM, White AP, Vernochet C, Schulz TJ, Xue R, Sass CA, Huang TI, Roberts-Toler C, Weiner LS, Sze C, Chacko AT, Deschamps LN, Herder LM, Truchan N, Glasgow AL, Holman AR, Gavila A, Hasselgren P-O, Mori MA, Molla M, Tseng Y-H: Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nat Med 2013; 19: 635–639.

Lidell ME, Betz MJ, Leinhard OD, Heglund M, Elander L, Slawik M, Mussack T, Nilsson D, Romu T, Nuutila P, Virtanen KA, Beuschlein F, Persson A, Boga M, Enerback S: Evidence for two types of brown adipose tissue in humans. Nat Med 2013; 19: 631–634.

Vire B, de Walque S, Restouin A, Olive D, Van Lint C, Collette Y: Anti-leukemia activity of MS-275 histone deacetylase inhibitor implicates 4-1BB/4-1BB immunomodulatory functions. PLoS One 2009; 4:e7085.

Buglio D, Mamidipudi V, Khashkely NM, Brady H, Heise C, Besterman J, Martell RE, MacBeth K, Younes A: The class-I HDAC inhibitor MGCD0103 induces apoptosis in Hodgkin lymphoma cell lines and synergizes with proteasome inhibitors by an HDAC6-independent mechanism. Br J Haematol 2010; 151: 387–396.

Christiansen AJ, West A, Banks KM, Haynes NM, Teng MW, Smyth MJ, Johnston RW: Eradication of solid tumors using histone deacetylase inhibitors combined with immune-stimulating antibodies. Proc Natl Acad Sci USA 2011; 108:4141–4146.

Kumari A, Cacan E, Greer S, Garnett-Benson C: Turning T cells on: epigenetically enhanced expression of effector T-cell costimulatory molecules on irradiated human tumor cells. J Immunother Cancer 2013; 1:1–16.

Kiskinis E, Chatzeli L, Curry E, Kaforou M, Frontini A, Cinti S, Montana G, Parker MG, Christian M: RIP140 represses the ‘brown-in-white’ adipocyte program including a futile cycle of triacylglycerol breakdown and synthesis. Mol Endocrinol 2014; 28: 344–356.

Kiskinis E, Hallberg M, Christian M, Olofsson M, Dilworth SM, White R, Parker MG: RIP140 directs histone and DNA methylation to silence Ucp1 expression in white adipocytes. EMBO J 2007; 26:4831–4840.