Biochemical Pharmacology 81 (2011) 838–847

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Decoy receptor 3: A pleiotropic immunomodulator and biomarker for inflammatory diseases, autoimmune diseases and cancer

Wan-Wan Lin [a], Shie-Liang Hsieh [b,c,d,e,*]

[a] Department of Pharmacology, National Taiwan University, Taipei, Taiwan
[b] Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan
[c] Institute and Department of Microbiology and Immunology, National Yang-Ming University, Taipei, Taiwan
[d] Immunology Research Center, National Yang-Ming University and Taipei Veterans General Hospital, Taipei, Taiwan
[e] Genomics Research Center, Academia Sinica, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 6 October 2010
Accepted 24 January 2011
Available online 2 February 2011

Keywords:
Inflammation
Autoimmune
Cancer
TNF

ABSTRACT

Recently, several decoy molecules belonging to tumor necrosis factor receptor superfamily (TNFRSF) have been identified, including decoy receptor 1 (DcR1), decoy receptor 2 (DcR2), and decoy receptor 3 (DcR3). One of the tumor necrosis factor superfamily (TNFSF) members, TNF-related apoptosis-inducing ligand (TRAIL), binds to DcR1 and DcR2, which are membranous receptors with a truncated cytoplasmic domain, thus unable to transduce TRAIL-mediated signaling. In contrast to DcR1 and DcR2, DcR3 is a soluble receptor capable of neutralizing the biological effects of three other TNFSF members: Fas ligand (FasL/TNFSF6/CD95L), LIGHT (TNFSF14) and TNF-like molecule 1A (TL1A/TNFSF15). Since FasL is a potent apoptosis- and inflammation-inducing factor, LIGHT is involved in apoptosis and inflammation, and TL1A is a T cell costimulator and is involved in gut inflammation, DcR3 can be defined as an immunomodulator on the basis of its neutralizing effects on FasL, LIGHT, and TL1A. Initial studies demonstrated that DcR3 expression is elevated in tumors cells; however, later work showed that DcR3 expression is also upregulated in inflammatory diseases, where serum DcR3 levels correlate with disease progression. In addition to its neutralizing effect, DcR3 also acts as an effector molecule to modulate cell function via 'non-decoy' activities. This review focuses on the immunomodulatory effects of DcR3 via 'decoy' and 'non-decoy' functions, and discusses the potential of DcR3 as a biomarker to predict cancer invasion and inflammation progression. We also discuss the possible utility of recombinant DcR3 as a therapeutic agent to control autoimmune diseases, as well as the potential to attenuate tumor progression by inhibiting DcR3 expression.

1. Discovery and characterisation of DcR3 gene and protein

Decoy receptor 3 (DcR3), also known as tumor necrosis factor receptor (TNFR) superfamily member 6b (TNFRSF6B)/TR6/M68, is a soluble receptor lacking a transmembrane domain and capable of neutralizing the biological effects of three members of the tumor necrosis factor superfamily (TNFSF): Fas ligand (FasL/CD95L/TNFSF6) [1], LIGHT [lymphotoxin-like, exhibits inducible expression, and competes with herpes simplex virus (HSV) glycoprotein D (gD) for HVEM, a receptor expressed by T lymphocytes/TNFSF14] [2], and TNF-like molecule 1A (TL1A/VEGI/TNFSF15) [3]. Early work demonstrated that the DcR3 gene was amplified in about half of 35 primary lung and colon tumors studied, with DcR3 messenger RNA being widely expressed in various malignant tissues [1,4].

The DcR3 gene is located at the extreme telomere of human chromosome 20 (20q13.3), and encodes a protein of 300 amino acids (NCBI accession #NM_032945). Western blot analysis of serum samples from transgenic mice overexpressing human full-length DcR3 revealed that it is a 33 kDa glycosylated protein. In addition, two alternatively spliced variants, DcR3v1 (NCBI accession #AAM94173) and DcR3v2 (NCBI accession #AAM94172), encode polypeptides of 74 amino acids and 139 amino acids, respectively. DcR3v1 is a truncated peptide comprising the N-terminal 48 and C-terminal 26 amino acids of DcR3. DcR3v2 contains the N-terminal 113 amino acids of DcR3, followed by 26 amino acids of unrelated residues. DcR3 is not found in mouse and rat genomes. However, the chicken (*Gallus gallus*) [5], rainbow trout (*Oncorhynchus mykiss*) [6], eel (*Conger myriaster*) [7] and banded dogfish (*Triakis scyllia*) [8] genomes contain DcR3-related

* Corresponding author at: Institute of Clinical Medicine, National Yang-Ming University, #155 Section 2, Li-Nong Sreet, Shih-Pai Chiu, Taipei, 112, Taiwan. Tel.: +886 2 28267161/28267227; fax: +886 2 28277933.
E-mail addresses: slhsieh@ym.edu.tw, slhsieh@vghtpe.gov.tw (S.-L. Hsieh).

0006-2952/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.01.011

genes to human DcR3, where all of these are secretory proteins with four cysteine-rich domains. In addition, DcR3-like sequences are present in frog (*Xenopus tropicalis*), Zebra finch (*Taeniopygia guttata*), chimpanzee (*Pan troglodytes*), and cattle (*Bos taurus*), suggesting DcR3 may be present in the genomes of amphibians, birds, and other mammals. In this article, we summarize current knowledge of DcR3, focusing on its roles in tumor progression, immunomodulation and inflammatory diseases, and discuss its clinical application as a biomarker for several diseases.

2. Decoy functions of DcR3 to cytokine ligands

Initial understanding of DcR3-mediated functions was derived from its interactions with three members of the TNFSF, i.e. FasL, LIGHT, and TL1A (Fig. 1), where the activities of DcR3 were hypothesized on the basis of the biological functions of these ligands. Recent review articles have discussed FasL [9], LIGHT [10,11], and TL1A [12,13] in detail; here we briefly summarize key features of FasL, LIGHT and TL1A before reviewing their relevance to DcR3 function. In particular, we focus on their abilities to modulate host immune responses, tumor growth, allergic and autoimmune responses, pathogen invasion, and other inflammatory reactions.

2.1. FasL

FasL is primarily produced by cytotoxic lymphocytes, i.e. natural killer (NK) cells and $CD8^+$ T cells, and contributes to the destruction of abnormal or infected cells. FasL is well known for its ability to induce apoptosis (programmed cell death) in target cells through binding to its receptor, Fas (Apo1/CD95) [9]. The extensive study of the FasL–Fas-mediated intracellular pathway, since its discovery in 1989, has established this system as a central regulator of apoptosis in mammals.

Fas, a type I transmembrane protein belonging to the TNFRSF, has a cysteine-rich extracellular domain that binds FasL, and an intracellular death domain responsible for transduction of the death signal that triggers apoptosis. When ligated by FasL (a trimeric protein), Fas induces the trimerization of Fas-associated death domain (FADD) protein leading to recruitment of procaspase-8, which subsequently undergoes autoproteolysis and becomes activated. This assembly of Fas, FADD and procaspase-8 results in the formation of a death-inducing signaling complex (DISC). Activated caspase-8 can, in turn, activate effector procaspase-3, -6, and -7 to induce apoptosis either directly via a process of transproteolysis in type I cells, or indirectly via proapoptotic molecules released from mitochondria in type II cells [14]. In type II cell apoptosis, caspase-8 can activate the proapoptotic Bcl-2 family member Bid, which then causes damage to the mitochondrial outer membrane, leading to release of cytochrome *c*. This, in turn, activates procaspase-9, leading to the activation of effector caspases, which degrade chromosomal DNA to cause cell death [9]. This Fas-mediated apoptotic pathway plays critical roles in the immune system, including the killing of pathogen-infected cells and of obsolete and potentially dangerous lymphocytes. Thus, Fas functions as a guardian against autoimmunity and tumor development. In contrast, dysregulation within the FasL–Fas system manifests in a severe impairment of the functional integrity and maintenance of immune homeostasis.

In addition to cell death, FasL can induce inflammatory reactions, cellular activation and proliferation in various cell types [9,15]. It has been demonstrated that Fas can trigger the expression of some inflammatory genes such as IL-6, MCP-1, IL-8, TNF-α, and IFN-γ in astrocytes, fibroblasts, synoviocytes, bronchial epithelial cells, keratinocytes, and vascular smooth muscle cells. Moreover, FasL can also recruit neutrophils to inflammatory sites and accelerate the acute inflammatory response. Therefore, the FasL–Fas interaction is suggested to play important roles in various inflammatory diseases such as hepatitis, acute rejection of graft transplant, graft-versus-host diseases (GVHD), rheumatoid arthritis (RA), and pulmonary fibrosis [9,16–18].

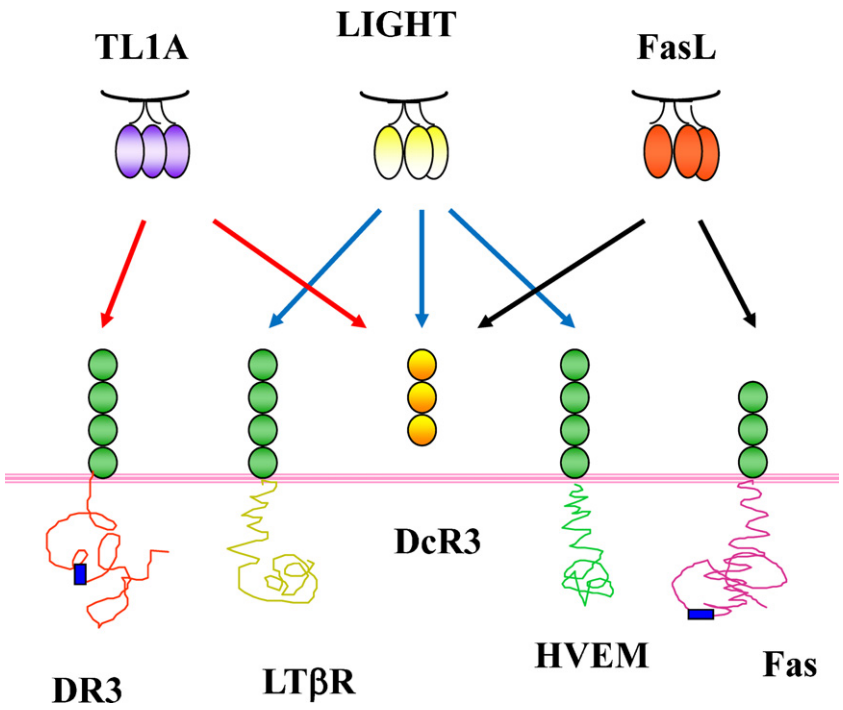

Fig. 1. Interaction of DcR3 and members of TNFRSF with FasL, LIGHT, and TL1A. DcR3 is able to compete the binding of FasL to Fas, thus prevent apoptosis. In addition, neutralization of LIGHT prevent the activation of LTbeta R and HVEM. DcR3 also blocks TL1A-mediated DR3 activation. Therefore, DcR3 is able to modulate cell function via blocking four members of TNFRSF simultaneously. Among these four membrane receptors, Fas and DR3 contain death domain (indicated as box in blue color) in the cytoplasmic regions.

2.2. LIGHT

LIGHT is predominantly expressed in lymphoid tissue and can bind three distinct members of the TNFRSF, i.e. the herpes virus entry mediator (HVEM/TNFRSF14) [19], lymphotoxin-β receptor (LTβR, TNFRSF3) [20] and DcR3 [2]. The functions of LIGHT are often cell-context specific due to its ability to interact with distinct receptors widely expressed by a variety of cell types.

LIGHT–LTβR-mediated signaling can promote immune responses via the activation of dendritic cells (DCs) and stromal cells. In this respect, induction of apoptosis, production of various cytokines, developmental organogenesis of lymph nodes, and restoration of secondary lymphoid structure and function emerge as major outcomes of the LIGHT–LTβR interaction [10,11]. LIGHT can also induce cell death in certain tumor cell lines through interaction with LTβR [21]. The anti-cancer activity of LIGHT was demonstrated by its ability to induce a cytolytic T-cell response leading to tumor rejection [22]. Thus, the LIGHT–LTβR axis is a potential target for controlling anti-tumor responses, and recent studies have provided encouraging new insights in this respect. Furthermore, it has been shown that disruption of the surface LIGHT–LTβR axis leads to defects of antiviral immunity and liver regeneration, but prevents autoimmune colitis and hyperlipidemia [23]. In most cases, the LIGHT–HVEM interaction selectively activates NF-κB, thereby initiating transcription of genes involved in cell survival, growth and inflammation [11]. These activities define LIGHT as a costimulatory molecule for T cells, which promotes enhanced proliferation, Th1-type cytokine production, and NF-κB translocation [11].

In addition to malignant diseases, LIGHT has emerged as a potential therapeutic target in inflammatory diseases of the mucosal, hepatic, joint and vascular tissues and the reproductive organs [11,24]. For example, there is compelling evidence for the involvement of the LIGHT–HVEM and LIGHT–LTβR pathways in atherogenesis. Expression of both LIGHT and HVEM is increased in activated monocytes and macrophages as well as in macrophages/foam cells within atherosclerotic plaques [25]. Moreover, HVEM can mediate the production of pro-atherogenic mediators by monocytes and endothelial cells, as well as macrophage migration and vascular smooth muscle cell proliferation. A recent study demonstrated that LIGHT expression on T cells results in hypertriglyceridemia, while inhibition of LIGHT signaling attenuates dyslipidemia in low-density lipoprotein receptor-deficient mice [26]. Additionally, LIGHT has been implicated in inflammatory bowel disease (IBD) [24], graft rejection [22], RA [27], and hepatic regeneration [28]. Thus, the LIGHT pathway is a target for the development of biologic-based therapeutics for autoimmune conditions, infectious diseases and cancer.

2.3. TL1A

TL1A is a novel TNF-like cytokine initially identified in endothelial cells and shown to be a ligand of death receptor-3 (DR3/Apo3/TNFRSF25). DR3 contains a death domain in its cytoplasmic tail and is preferentially expressed by lymphocytes and endothelial cells. Its activation by TL1A can not only induce cell apoptosis, by activating the caspase cascade through interaction with TRADD and FADD [12,13], but also activate multiple cell survival signaling pathways including NF-κB, STAT3, JNK, p38 MAPK and ERK [12,13].

Through binding to DR3 endogenous TL1A can suppress endothelial cell proliferation and angiogenesis in an autocrine manner [29]. This anti-angiogenic action of TL1A is evidenced by the inhibition of tumor neovascularization and progression in a mouse xenograft tumor model [30]. Recent studies indicated that TL1A can be induced not only in endothelial cells, but also in monocytes, macrophages, DCs, T lymphocytes and chondrocytes in response to stimulation with immune complexes, Toll-like receptor ligands, inflammatory cytokines, and T-cell receptor activator [13]. TL1A can also bind DR3 on immune cells, where this interaction leads to activation and proliferation of lymphocytes and NK cells, and skews T-cell differentiation in favor of Th1 and Th17 subsets [3,12,13]. Moreover, TL1A has been shown to suppress the proliferation of tumor cells and induce maturation of DC and osteoclasts [12].

There is growing evidence that TL1A/DR3-activated signaling cascades may participate in the pathogenesis of IBD [31] and RA [32]. Increased expression of TL1A and DR3 is observed in both ulcerative colitis and Crohn's disease [31], and upregulation of TL1A correlates with the severity of inflammation in IBD. In an animal model of IBD, TL1A–DR3 interaction was found to exacerbate intestinal inflammation via stimulation of Th1 and Th17 pathways [33]. Notably, genetic studies have identified associations between TL1A alleles and Crohn's disease [34].

2.4. Decoy function of DcR3

The ability of DcR3 to act as a decoy receptor to neutralize the bioactive cytokines FasL, LIGHT and TL1A gives it the potential to modulate host immunity by inhibiting the activation of the TNFRSF members Fas, HVEM, LTβR, and DR3 (Fig. 2). The majority of early studies on DcR3-mediated immunosuppression have focused on its interaction with FasL; more recently, its binding to LIGHT and TL1A have been of interest. In particular, the biological functions of DcR3 have been unmasked in the context of cancer. It was speculated that tumor cells expressing high levels of DcR3 might be able to evade cytotoxic attack by inhibition of the FasL–Fas interaction, which is known to induce apoptosis [35]. In support of this, Roth et al. showed that soluble DcR3 is expressed by malignant gliomas and can suppress FasL-induced apoptosis and chemotaxis [36]. Furthermore, Connolly et al. showed that injection of recombinant DcR3 attenuates FasL-mediated fulminant hepatic apoptosis and blocks FasL-induced cell death in mice [37]. The resistance of human pancreatic cancer cells to FasL is likely due, at least in part, to the presence of high levels of DcR3 protein; this resistance can be overcome by treatment with DcR3-specific small interfering RNA [38,39]. Thus, targeted reduction of endogenous DcR3 levels represents a novel therapeutic strategy in cancer. Even though DcR3 can protect tumors from attack by $FasL^+CD4^+$ cells, it is unable to guard against cytotoxicity mediated by granzyme and perforin, the two potent apoptotic-inducing factors secreted by $CD8^+$ T cells. This observation challenges the idea that DcR3 can help tumor cells to escape from T-cell-mediated cytotoxicity, given that $CD8^+$ T cells are more potent tumor cell killers than $CD4^+$ T cells.

With regard to LIGHT and TL1A, the results of several studies indicate that DcR3 can dampen T-cell responses to alloantigens by blocking the LIGHT–HVEM interaction [40]. Moreover, DcR3 can block LIGHT- and TL1A-induced islet and cancer cell apoptosis [2,41]. Since TL1A also enhances the responsiveness of T cells to IL-2 and increases their secretion of proinflammatory cytokines, it has been speculated that DcR3 might attenuate T-cell activation via its action as a decoy receptor for TL1A [3]. Furthermore, DcR3 has been reported to neutralize endogenous TL1A secreted by human umbilical vein endothelial cells, leading to endothelial cell migration, proliferation, differentiation, MMP-2 activation and angiogenesis. This suggests that DcR3 might promote angiogenesis by antagonizing the autocrine angiostatic effect of TL1A on endothelial cells [29].

3. Non-decoy actions of DcR3

In addition to neutralizing TL1A, LIGHT and FasL, DcR3 also acts as an effector molecule to modulate the activities of many cell types directly (Fig. 2).

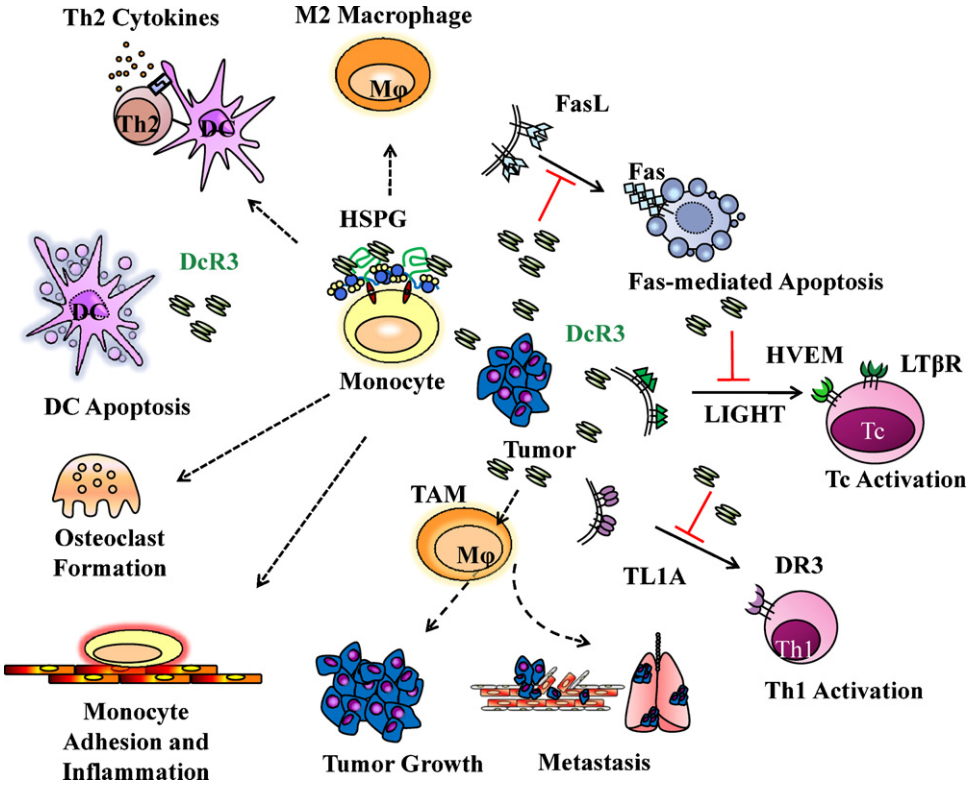

Fig. 2. DcR3 is a secretory protein of tumor cells, and can modulate immune response and help tumor progress from different action context. In one hand, DcR3 functions as a decoy receptor for FasL, LIGHT and TL1A, three major cytokines contributing to the T-cell-mediated host immune surveillance, and allows cancer cells escape from T-cell-mediated cytotoxic effect. On the other hand, DcR3 also functions as an effector by cross-linking HSPG present on plasma membranes to skew macrophage differentiation into M2 phenotypes, regulate DC differentiation to Th2 polarization, induce DC apoptosis, drive monocyte differentiation into osteoclast, and increase monocyte adhesion. Both decoy and non-decoy actions of DcR3 facilitate tumor progress and contribute to the pathogenesis of diseases.

3.1. Regulation of DC differentiation leading to Th2 polarization

Soluble DcR3.Fc can bind to $CD14^+$ monocytes and modulate their differentiation and maturation into DCs [42]. The expression of human leukocyte antigen (HLA)-DR, CD1a, CD40, CD54, and CD80/B7.1 was shown to be reduced in DcR3.Fc-treated DCs. However, the expression of CD86/B7.2 was upregulated. Moreover, the proliferation of $CD4^+$ T cells co-cultured with DcR3.Fc-treated DCs was skewed in favor of a Th2-predominant phenotype, indicating that DcR3 has a Th1-suppressing effect [42]. Since none of DcR3's known ligands were detected in this assay system, the DcR3-mediated effect observed cannot be attributed to its neutralizing effect on FasL, LIGHT or TL1A. The Th1-suppressive effect of DcR3 was further demonstrated both *in vitro* and *in vivo* using transgenic mice [43]. Stimulation of splenocytes from DcR3-transgenic animals with influenza hemagglutinin$_{126–138}$ peptide caused elevated production of IL-4 and IL-10 and downregulation of IFN-γ, IL-12, and TNF-α compared to controls, while mice infected with *Listeria monocytogenes* showed attenuated expression of IFN-γ and increased susceptibility to infection. This Th2 cell-biased phenotype was attributed to decreased IL-2 secretion by T cells [43].

3.2. Regulation of M2 macrophage and TAM differentiation

In vitro studies have demonstrated that DcR3 can drive macrophages towards an M2 phenotype, where DcR3 downregulates the expression of CD14, CD16 and HLA-DR, but upregulates the expression of CD206 (mannose receptor). Moreover, phagocytic activity towards immune complexes and apoptotic bodies, as well as the production of free radicals and proinflammatory cytokines in response to lipopolysaccharide, is impaired in DcR3-treated macrophages [44]. Microarray analysis further revealed that DcR3-treated macrophages display the characteristics of tumor-associated macrophages (TAMs), such as the upregulation of CD206, arginase-1 (M2-specific markers), M160, IL1RN, IL1R2, CCL22, MMP-7 and MMP-9, alongside downregulation of iNOS, CD16/32/64, CD80, IL-1, IL-6 and CLCL2 [45]. This suggests that DcR3 might have potent suppressive effects on the host immune system, and thus favor tumor growth *in vivo*.

3.3. Induction of actin reorganization and adhesion in monocytes

DcR3 can induce actin reorganization in human monocytes and has also been shown to trigger multiple signaling molecules, such as PKC, phosphatidylinositol 3-kinase (PI3K), focal adhesion kinase and Src kinases, thereby promoting cell adhesion [46]. Furthermore, DcR3 induces NF-κB-mediated expression of ICAM-1, VCAM-1 and IL-8 by monocytes such that their binding to endothelial cells is enhanced [47]. A recent study further showed that DcR3.Fc acted on both THP-1 monocytes and differentiated macrophages to increase their expression of integrin α4, thus promoting cell aggregation and proliferation and reducing apoptosis [48]. Since DcR3 is upregulated in cancer cells, these observations suggest that it may play important roles in modulating the migration and trafficking of monocytes/macrophages in the tumor microenvironment.

3.4. Induction of osteoclast differentiation and alteration of bone homeostasis

DcR3 induces osteoclast formation from human monocytes, murine macrophages, and bone marrow cells by promoting their differentiation into polymorphonuclear giant cells that express

TRAP, CD51/61 and MMP-9 and display bone resorptive activity [49]. Since DcR3-mediated osteoclast differentiation is abrogated by TNFR.Fc, and DcR3 treatment can induce secretion of the osteoclastogenic cytokine TNF-α through ERK and p38 MAPK signaling pathways, DcR3-induced TNF-α synthesis is implicated as a critical regulator of osteoclast formation. Enhancement of osteoclast differentiation *in vitro* was confirmed by altered bone homeostasis in DcR3-transgenic mice. Bone mineral density (BMD) and bone mineral content (BMC) were significantly lower in DcR3-transgenic mice as compared to wild-type controls, where this was associated with increased osteoclast numbers [50]. In addition, local administration of DcR3 into the metaphysis of the tibia significantly decreased the BMD and BMC, with increases in both osteoclast numbers and the resorptive activity of mature osteoclasts. These results indicate that DcR3 may play an important role in diseases such as osteoporosis and might also contribute to bone erosion in cancer patients.

3.5. Sensitization of cells to TRAIL-induced apoptosis

DcR3 sensitizes Jurkat and U937 cells to apoptosis induced by the TNF-related apoptosis-inducing ligand (TRAIL), another member of TNFSF and with anti-cancer potential. Nevertheless, DcR3 by itself does not stimulate apoptosis, nor affect cell death triggered by Fas or TNF-α. The ability to augment TRAIL-induced cell death has not been observed for either soluble LTβR or soluble DR3, indicating that binding to LIGHT or TL1A is, in itself, insufficient to trigger TRAIL sensitivity. Incubation with DcR3 did not increase the surface expression of the TRAIL receptor, and the levels of FADD protein and cellular FLICE-like inhibitory protein (FLIP) were not altered. Instead, TRAIL engagement in the presence of DcR3 resulted in increased activation of caspase-8, elevated cleavage of Bid, and enhanced release of Smac and cytochrome *c* from mitochondria into the cytosol compared to TRAIL alone. This, in turn, led to increased activation of caspase-9 and caspase-3 [51].

In addition to sensitizing T cells to TRAIL-mediated apoptosis, a high dose of DcR3 (10 μg/ml) induces DCs, but not macrophages, to undergo apoptosis; this is due to activation of PKC and JNK, which subsequently leads to elevated DR5 expression and, hence, recruitment of FADD. The association of FADD with DR5 results in the formation of a DISC, where activation of caspase-2 and caspase-8 triggers the downstream apoptotic signaling cascade [52]. The ability of DcR3 to induce T cell and DC apoptosis could potentially be harnessed to suppress host immunity during treatment of autoimmune diseases.

3.6. Effector action via heparin sulfate proteoglycans

Several studies have demonstrated that the pleiotropic effects of DcR3 on immunomodulation, cell adhesion, and the differentiation of antigen-presenting cells are independent of its interaction with its TNFR-like ligands, FasL, LIGHT and TL1A. A glycosaminoglycan (GAG)-binding domain located in the DcR3 C-terminal region is able to bind and cross-link heparan sulfate proteoglycans (HSPG), such as syndecan 2 and CD44v3. DcR3-HBD.Fc, a fusion protein comprising the GAG-binding region of DcR3 with an Fc fragment of human IgG1, has similar activity to DcR3.Fc with regard to the activation of PKC and the induction of monocyte adhesion and DC apoptosis, even though it is unable to interact with LIGHT, FasL, or TL1A [52,53]. Although the Fc component of this fusion protein stabilizes dimeric DcR3 and, thus, may enhance cross-linking activity, it should be noted that transgenic mice overexpressing DcR3 also exhibit attenuated Th1 differentiation [43] and enhanced osteoclast differentiation [50]. This indicates that the biological effects of DcR3 *in vivo* are not restricted to its neutralizing effects on FasL, LIGHT, and TL1A. Together these data define DcR3 as a pleiotropic effector, which can regulate cellular functions through interactions with HSPG. It is likely that the identification of additional HSPG ligands for DcR3 will lead to the elucidation of yet more effector functions.

3.7. Inhibition of MHC-II expression via epigenetic regulation

One of the key characteristics of TAMs is the downregulation of MHC-II molecules, but the mechanism that underlies this has not yet been fully elucidated. Among the DcR3-modulated genes expressed by TAMs, those that encode proteins involved in MHC-II-dependent antigen presentation are downregulated substantially, together with the class II transactivator (CIITA), the master regulator of MHC-II expression [45]. ERK- and JNK-induced deacetylation of histones associated with the CIITA promoter was found to be responsible for the DcR3-mediated down-regulation of MHC-II expression. Furthermore, the level of DcR3 expression by cancer cells correlated inversely with HLA-DR levels on TAMs and with the overall survival time of patients with pancreatic cancer. The role of DcR3 in the development of TAMs was further confirmed using transgenic mice overexpressing DcR3. This study revealed the molecular mechanism underlying impaired MHC-II-mediated antigen presentation by TAMs, and raised the possibility that reversal of TAM-induced immunosuppression via inhibition of DcR3 expression might represent a target for the design of new therapeutics.

4. DcR3 as a biomarker in cancer progression

There is accumulating evidence that DcR3 is expressed by tumor cells originating from various lineages, including adenocarcinomas of the esophagus [54], stomach [55], colon, rectum [1], pancreas [56], lung [1], glioblastoma multiforme [36,57], renal cell cancer (RCC) [58], ovarian cancer [59], virus-associated lymphomas [60], diffuse large B-cell lymphoma [61], multiple myeloma [62], oral cancer [63], hepatocellular carcinoma [64], and chronic liver diseases, which frequently lead to cancer formation [65]. The upregulation of DcR3 by these cells has been demonstrated by immunohistochemical staining [54,63], tissue microarrays [64], in situ hybridization [65] and analysis of serum samples from cancer patients [58,62,63,66–68].

It has become apparent that elevated levels of DcR3 in serum or tissues correlate with poor prognosis and/or resistance to treatment in some cancer patients. This includes gastric carcinomas [55], esophageal adenocarcinoma [54], RCC [58], ovarian cancer [59,68], colorectal cancer [69], hepatocellular carcinoma [64,70], pancreatic cancer [38], and oral cavity cancer [63]. In the case of esophageal adenocarcinoma, significantly more overexpression of DcR3 was observed in esophagogastrectomy specimens containing areas of high-grade dysplasia or carcinoma than in low-grade dysplasia, benign esophageal mucosa or Barrett esophagus [54]. Likewise, high levels of DcR3 expression are associated with high-grade and high-stage RCCs. DcR3 expression correlates positively with the incidence of distant metastasis and lymph node metastasis, but negatively with disease-specific survival [58]. In oral cavity cancer, elevated serum DcR3 is also associated with nodal metastasis and poor prognosis [63]. Analyses of DNA isolated from recurrent oral tumors [63], glioblastoma [57] and gastric adenocarcinoma [71] have confirmed that increased DcR3 copy-number is common in cancer patients. In addition, DcR3 expression levels correlate with impaired differentiation of colorectal carcinoma cells, lymph node metastasis, and pathological stage [64]. A genotyping study indicated that a DcR3 promoter polymorphism, C147T, is associated with a significantly elevated risk of esophageal squamous cell carcinoma in the

Chinese Han population [72]. Although substantial amplification of DcR3 gene has been observed in patients with myelodysplastic syndrome patients, the relevance of this to disease progression remains unclear [73].

5. DcR3 as a biomarker for autoimmune and inflammatory diseases

DcR3 is not detectable in most normal tissues, but its expression is upregulated in response to some pathogens or insults. In particular DcR3 levels are elevated in systemic inflammatory diseases, where serum concentrations are significantly increased in silicosis [74], bacterial infections [75], Crohn's disease [76], active ulcerative colitis [31], systemic lupus erythematosus [77], atopic dermatitis [78], experimental autoimmune encephalomyelitis (EAE) [79], RA [32], kidney diseases [80] and acute respiratory distress syndrome (ARDS) [81]. Chen et al. demonstrated that high plasma levels of DcR3 correlate with the development of multiple-organ dysfunction and can independently predict 28-day mortality in patients with ARDS. Therefore, serum DcR3 is a valuable marker in predicting the outcome of inflammatory diseases [81].

6. Regulation of DcR3 expression in cancer cells and inflammatory reactions

6.1. Upregulation of DcR3 by Epstein–Barr virus

Although DcR3 expression is known to be upregulated in cancer cells, its regulation is not well understood. It has been reported that DcR3 is overexpressed in Epstein–Barr virus (EBV)-associated lymphoma, but not in reactive lymphadenopathy [60]. This suggests that EBV might use DcR3 to evade the immune system during lymphomagenesis, or that virus-infected lymphoma cells expressing DcR3 might be selected during multistep tumorigenesis. In this regard, the EBV transcription activator Rta has been shown to bind to the DcR3 promoter and upregulate DcR3 expression [82]. Moreover, EBV latent membrane protein 1 has been shown to promote DcR3 expression via NF-κB- and PI3K-mediated signaling events [83]. This demonstrates the ability of virus-encoded transcription factors to upregulate DcR3 expression in cancer cells.

6.2. Upregulation of DcR3 by NF-κB

Recent studies have demonstrated that inflammation is a key factor in the induction of DcR3 expression. For example, increased expression of DcR3 by appendix epithelia from patients with acute appendicitis [84] and by the epithelial layer of ileum specimens from patients with Crohn's disease [76] has been observed.

In vitro studies have confirmed the abilities of intestinal epithelial cells to express DcR3 in response to lipopolysaccharide (LPS) or TNF-α stimulation [76,85], where mechanistic studies using intestinal epithelial cells [76] and human pancreatic cancer AsPC-1 cells [39] have identified NF-κB activation as being essential for DcR3 expression. In addition, Toll-like receptor-induced DcR3 release from human intestinal epithelial cell lines appears to be via the activation of ERK1/2, JNK, Src-like protein tyrosine kinases, PI3K, and the transcription factor NF-κB [84]. Immune cells, such as monocytes and macrophages, have also been shown to express DcR3 following chronic exposure to silica [74] or bacterial antigens [75], and DcR3 is upregulated in lamina propria T cells from the inflamed mucosa of ulcerative colitis patients [85]. Tumorigenesis is typically associated with chronic inflammation, where upregulation of DcR3 expression might further amplify the inflammatory response via its non-decoy effects on immune cells, thus promoting a microenvironment permissive tumor growth.

6.3. DcR3 expression by keratinocytes

DcR3 mRNA is constitutively expressed at high levels by cultured human keratinocytes, with a rapid decrease in expression upon exposure to UVB irradiation [86]. This suggests that DcR3 may play an important homeostatic role within the epidermis. In eel keratinocytes LPS treatment promotes increased expression of DcR3 [7].

6.4. DcR3 expression in genital organs

As well as contributing to the host response to inflammatory insults, DcR3 has been suggested to play a role in follicular and placental development in vertebrate ovaries. In the brook trout DcR3 mRNA was detected in the ovary, where its expression by granulosa cells is substantially upregulated by phorbol ester stimulation, and in the testes. Moreover, trout DcR3 mRNA levels undergo cyclical changes during natural ovulation; expression is upregulated during the 24 h period immediately post-ovulation and then progressively downregulated [87]. In humans, serum DcR3 levels also exhibit cyclical changes during the menstrual cycle, tending to be highest during the mid-luteal phase, while DcR3 expression by cultured human endometrial cells is upregulated in response to estrogen and progesterone in a dose-dependent manner. The observation that DcR3 protein levels are reduced in the endometria of anembryonic pregnancies suggests an active role for DcR3 in the maintenance of successful pregnancies [88]. Another study reported reduced levels of DcR3 mRNA in granulosa cells from atretic ovarian follicles compared to healthy follicles, where this might contribute to granulosa cell apoptosis during atresia [89]. A potential role for DcR3 in placental development was revealed by immunohistological studies, which identified cell-specific and temporally programmed expression patterns for LIGHT and its receptors, DcR3 and LTβR. DcR3, LIGHT and LTβR are present in human syncytiotrophoblast and cytotrophoblast cells during the early (6–7 weeks) and early-to-middle (8–13 weeks) phases of gestation. In contrast, these proteins were detected in placental stromal cells only from week 8 onwards. These results suggest that the LIGHT–DcR3–LTβR system may regulate the early-to-middle stages of placental development, and may play a role in preserving the immune privilege of the placenta [90]. Since mice do not express DcR3, these potential functions of DcR3 could be tested *in vivo* by comparison of wild-type and DcR3-transgenic mice; these studies might shed light on whether recombinant DcR3 might be utilized to enhance the rate of successful pregnancy in the future.

7. DcR3 as a potential therapeutic agent

7.1. Type 1 diabetes

Given that type I diabetes is a Th1-mediated autoimmune disease, DcR3-mediated Th1 suppression becomes a new strategy for this condition. *In vivo* and *in vitro* studies support the notion that DC-derived Th1 activity contributes to disease progression, where DcR3 might limit this by modulating DC differentiation [91,92]. DcR3.Fc treatment of bone-marrow-derived DCs from nonobese diabetic (NOD) mice, the prototype animal model of type I diabetes, leads to upregulation of CD86 and downregulation of CD80, suggesting a skewing of the T-cell response towards the Th2 phenotype. Consistent with this, DcR3.Fc-treated DCs significantly reduced the proliferation of $CD4^+$ T cells and the secretion of IFN-γ from T cells as compared to untreated DCs [90]. In adoptive transfer experiments, NOD mice receiving DcR3.Fc-treated DCs and autoreactive T cells exhibited delayed onset of diabetes and a decrease in diabetic severity compared to mice that received normal DCs and T cells [91].

The failure of islet transplantation for type I diabetes therapy primarily results from either islet primary nonfunction (PNF) or graft rejection. PNF is defined as the loss of islet function after transplantation for reasons other than graft rejection, and is an obstacle to successful and efficient islet transplantation. In a study using NOD mice, where DcR3.Fc was administered intraperitoneally twice a day at 15 mg/kg/day, commencing immediately after allogeneic islet transplantation, PNF (defined as blood glucose >18 mM at 48 h post-transplantation) was totally prevented [91]. This led Wu et al. to claim that DcR3 is an effective inhibitor of Fas-mediated islet PNF. LIGHT and TL1A, like Fas, are capable of inducing islet cell apoptosis, and this suggests an alternative mechanism through which DcR3 might protect islets [41]. The ability of DcR3 to prevent chronic graft rejection was also observed in transgenic mice, where islet-specific expression of DcR3 protected mice from autoimmune and cyclophosphamide-induced diabetes [93]. Moreover, the transgenic islets were associated with higher transplantation success rates and survived longer than wild-type islets. These findings indicate that both local DcR3 expression and systemic administration of DcR3 plasmid or protein can significantly inhibit insulitis and diabetes. Finally it is interesting to note that lymphocytes from DcR3.Fc-treated mice display reduced proliferative potential and can ameliorate diabetes by adoptive transfer [92].

Taken together, current data support the potential of DcR3 as a therapeutic agent for type 1 diabetes. Importantly, islet-specific expression of DcR3 does not alter the integrity of islets, affect systemic immune responses or promote tumor cell growth [93]. Therefore, genetic manipulation of beta cells to promote DcR3 expression could improve the success and longevity of islet transplants.

7.2. Organ transplantation

Graft rejection is still a major barrier to successful outcomes in transplantation surgery. New strategies to reduce T-cell-mediated immunoreactivity, prolong the lifespan of graft function and minimize systemic side effects are an important requirement. The immune-evasive and Th2-promoting properties of DcR3, described above in the context of type 1 diabetes, might also confer benefits by prolonging xenograft survival other than islet transplantation [94]. Indeed, DcR3.Fc can suppress the production of lymphokines (IFN-γ and GM-CSF) by alloantigen-stimulated mouse T cells *ex vivo*, inhibit cytotoxic T lymphocyte development *in vitro*, and ameliorate the rejection of heart allografts *in vivo* [40].

7.3. Rheumatoid arthritis

RA is a chronic inflammatory disease with an autoimmune etiology and complex pathogenesis that involves synovial cell proliferation and fibrosis, pannus formation, and cartilage and bone erosion. This disease process is mediated by an interdependent network of proinflammatory cytokines. Recently, compelling evidence emerged to suggest that DcR3 might be able to attenuate joint inflammation by neutralizing TL1A, LIGHT, and FasL, where all three of these ligands have been shown to play critical roles in the pathogenesis of collagen-induced arthritis (CIA) in mice.

It has been reported that TL1A aggravates CIA, with TL1A administration leading to the occurrence of multiple enlarged germinal centers in the spleen, and elevated serum anti-collagen Ab titers. TL1A also augments TNF-α production by T cells as a consequence of T-cell receptor ligation, and greatly enhances Th17 differentiation and IL-17 production [95]. In contrast, treatment with an antagonistic anti-TL1A mAb protects animals from CIA [96]. LIGHT also plays a role in immunopathogenic conditions that are associated with bone loss, e.g. it has been shown to induce proliferation and inflammatory responses in RA synovial fibroblasts via LTβR [97]. Prophylactic treatment with an inhibitor of LIGHT, LTβR-Ig, was shown to block the induction of CIA in mice and adjuvant arthritis in Lewis rats. Treatment with LTβR-Ig also ablated follicular DC networks in the draining lymph nodes, suggesting that impaired class switching and affinity maturation might be the cause of decreased levels of pathological autoantibodies [97]. In addition to TL1A and LIGHT, activation of the FasL–Fas system was reported to trigger a proinflammatory response in rheumatoid synoviocytes [18]; this is likely due to activation of NF-κB and AP-1, and expression of genes, such as MMPs and chemokines [18].

7.4. Kidney disease

DcR3 has been identified as a novel biomarker for disease progression in patients with chronic kidney disease. DcR3 levels in serum are significantly increased in autoimmune crescentic glomerulonephritis, and administration of recombinant DcR3.Fc can prevent the development of this disease [80]. This beneficial effect might be due to modulation of T-cell activation/proliferation or B-cell activation, protection against apoptosis or suppression of mononuclear leukocyte infiltration into the kidney.

7.5. Multiple sclerosis

It has been reported that administration of recombinant DcR3.Fc fusion protein has a significant ameliorative effect on EAE symptoms as evidenced by a lower clinical score and reduced inflammation in the spinal cord. Expression of TNF-α, IFN-γ, and IL-17 was found to be diminished in the spinal cords in DcR3-treated mice [79], while there was a dramatic reduction in IL-17-producing $CD4^+$ T cells and increased numbers of IL-4-producing $CD4^+$ T cells in the central nervous system. Moreover, myelin oligodendrocyte glycoprotein-specific T-cell proliferation was significantly inhibited in DcR3-treated mice, while adoptive transfer studies demonstrated that splenocytes from DcR3-treated mice retained their disease-inhibiting ability [79]. This suggests that DcR3 is able to suppress encephalitogenic Th17 cells, and has potential as a treatment for human multiple sclerosis. This is in line with previous observations that treatment of proteolipid protein-specific lymph node cells with DcR3.Fc protein resulted in suppression of IFN-γ and IL-17, reduced numbers of Th17 cells and diminished encephalitogenicity [98].

7.6. Inflammatory bowel disease

There is evidence for the association of the DcR3–TL1A–LIGHT system with IBD. This common inflammatory disorder has a complex etiology involving both genetic and environmental triggers, including defects in the mucosal barrier, as well as persistent dysregulation of the immune response to commensal bacteria in the intestine. A genome-wide study identified loci on chromosomes 20q13, close to the DcR3 gene, as being significantly associated with IBD [99]. In addition, TL1A [31] and LIGHT [24] have been described as pathological mediators of IBD. Therefore, DcR3.Fc may have beneficial effects on IBD via neutralizing TL1A and LIGHT. It would also be interesting to test whether aberrant expression of DcR3 correlates with the onset and severity of IBD.

7.7. The therapeutic potential of DcR3 is limited by its instability

As with all biologics, the *in vivo* stability of DcR3 protein will determine its efficacy as a therapeutic agent. Previous studies showed that, following subcutaneous administration in primates and mice, DcR3 is rapidly degraded to a major circulating fragment,

DcR3(1–218) [100]. This metabolic product retains its affinity for LIGHT, but no longer binds to FasL. However, when DcR3 was modified by changing the arginine residue at position 218 to glutamine to generate DcR3(R218Q), its half-life following intravenous administration was prolonged and its function as a decoy receptor for LIGHT and FasL was retained [100]. Therefore, the feasibility of utilizing DcR3 as a therapeutic agent will depend on the success of strategies to increase its stability, while maintaining its binding specificity for FasL, LIGHT and TL1A, as well as preventing immunogenicity to host immune system.

8. Conclusions and perspectives

DcR3 is a novel immunosuppressant whose biological functions result in part from its ability to neutralize the activities of TL1A, LIGHT and FasL, as well as from non-decoy functions. Since soluble DcR3 levels will likely affect the homeostasis of cells and tissues, understanding the regulation of DcR3 expression in specific pathophysiological conditions might provide important insights into disease progression and treatment.

The overexpression of DcR3 protein might provide an important biomarker, which could aid in the diagnosis, e.g. of high-grade carcinoma. Indeed, DcR3 has been identified as an indicator of disease prognosis, which may result from the potent effect of DcR3 in skewing macrophages into TAM and, thus, promoting tumor growth and invasion Therefore, monitoring DcR3 expression may be useful for the diagnosis of cancers as well as for the determination of differentiation, metastasis, and carcinoma stage.

Within the TNFR family, DcR3 is the only member capable of neutralizing three ligands. Therefore, recombinant DcR3.Fc has a substantial impact on inflammatory processes. It is noteworthy that mouse and rat do not express DcR3, although both species express FasL, LIGHT, and TL1A and respond to treatment with human DcR3. In humans, DcR3 is upregulated in pathological conditions such as cancer, autoimmune and inflammatory diseases; this, therefore, raises a great concern as to how well murine models of these conditions reflect what is happening in humans, especially in diseases involving FasL, LIGHT, and TL1A.

Although the importance of DcR3 in the regulation of human disease has been revealed in recent years, several hurdles must be overcome to fully understand this mysterious molecule. First, how DcR3 modulates host immunity via 'non-neutralizing' effect is still unclear. We have shown that DcR3 is able to induce macrophage differentiation and trigger signaling via cross-linking GAGs. In addition, DcR3_HBD.Fc, a fusion protein comprising the GAG-binding region of DcR3 with an Fc fragment, has the same effect as DcR3.Fc in activating PKC and inducing cell adhesion [53]. However, there still is a gap between HSPG activation and the triggering of downstream signaling cascades. The second challenge is to understand the regulation of DcR3 expression, where DcR3 is almost undetectable in non-pathological conditions, but is highly upregulated in tumor cells from various origins. Thus, DcR3 might be upregulated by common factors that are activated in cancer cells from different lineages. Since DcR3 has potent suppressive effects on host immunity, inhibiting its expression might be beneficial in the treatment of cancers. On the other hand, turning on DcR3 expression may help to control the onset of autoimmune disease and other inflammatory conditions.

Acknowledgements

The authors greatly acknowledge Miss Hsin-Chuan Chang for technical assistance, and the financial support of the National Research Program for Genomic Medicine, National Science Council, Taiwan (NSC 99-2811-B-010-018, NSC 99-3112-B-010-011, NSC98-2320-B-002-010-MY3 and NSC 98-2320-B-010-022-MY3), the National Yang-Ming University, Taiwan (96A-D-D132 from MOE), and Taipei Veterans General Hospital (V97S5-001).

References

[1] Pitti RM, Marsters SA, Lawrence DA, Roy M, Kischkel FC, Dowd P, et al. Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. Nature 1998;396:699–703.
[2] Yu KY, Kwon B, Ni J, Zhai Y, Ebner R, Kwon BS. A newly identified member of tumor necrosis factor receptor superfamily (TR6) suppresses LIGHT-mediated apoptosis. J Biol Chem 1999;274:13733–6.
[3] Migone TS, Zhang J, Luo X, Zhuang L, Chen C, Hu B, et al. TL1A is a TNF-like ligand for DR3 and TR6/DcR3 and functions as a T cell costimulator. Immunity 2002;16:479–92.
[4] Bai C, Connolly B, Metzker ML, Hilliard CA, Liu X, Sandig V, et al. Overexpression of M68/DcR3 in human gastrointestinal tract tumors independent of gene amplification and its location in a four-gene cluster. Proc Natl Acad Sci USA 2000;97:1230–5.
[5] Bridgham JT, Johnson AL. Characterization of chicken TNFR superfamily decoy receptors. DcR3 and osteoprotegerin. Biochem Biophys Res Commun 2003;307:956–61.
[6] Liu L, Fujiki K, Dixon B, Sundick RS. Cloning of a novel rainbow trout (*Oncorhynchus mykiss*) CC chemokine with a fractalkine-like stalk and a TNF decoy receptor using cDNA fragments containing AU-rich elements. Cytokine 2002;17:71–81.
[7] Tsutsui S, Yoshino Y, Matsui S, Nakamura O, Muramoto K, Watanabe T. Isolation of epidermal cells and cDNA cloning of TNF decoy receptor 3 of conger eel, *Conger myriaster*. Fish Shellfish Immunol 2008;24:366–71.
[8] Inoue Y, Morinaga A, Takizawa F, Saito T, Endo M, Haruta C, et al. Molecular cloning and preliminary expression analysis of banded dogfish (*Triakis scyllia*) TNF decoy receptor 3 (TNFRSF6B). Fish Shellfish Immunol 2008;24:360–5.
[9] Strasser A, Jost PJ, Nagata S. The many roles of FAS receptor signaling in the immune system. Immunity 2009;30:180–92.
[10] Ware CF. Targeting lymphocyte activation through the lymphotoxin and LIGHT pathways. Immunol Rev 2008;223:186–201.
[11] Ware CF. Targeting the LIGHT–HVEM pathway. Adv Exp Med Biol 2009;647:146–55.
[12] Sethi G, Sung B, Aggarwal BB. Therapeutic potential of VEGI/TL1A in autoimmunity and cancer. Adv Exp Med Biol 2009;647:207–15.
[13] Bayry J. Immunology: TL1A in the inflammatory network in autoimmune diseases. Nat Rev Rheumatol 2010;6:67–8.
[14] Barnhart BC, Alappat EC, Peter ME. The CD95 type I/type II model. Semin Immunol 2003;15:185–93.
[15] Whiteside TL. The role of death receptor ligands in shaping tumor microenvironment. Immunol Invest 2007;36:25–46.
[16] Kondo T, Suda T, Fukuyama H, Adachi M, Nagata S. Essential roles of the Fas ligand in the development of hepatitis. Nat Med 1997;3:409–13.
[17] Kuwano K, Hagimoto N, Kawasaki M, Yatomi T, Nakamura N, Nagata S, et al. Essential roles of the Fas–Fas ligand pathway in the development of pulmonary fibrosis. J Clin Invest 1999;104:13–9.
[18] Palao G, Santiago B, Galindo MA, Rullas JN, Alcami J, Ramirez JC, et al. Fas activation of a proinflammatory program in rheumatoid synoviocytes and its regulation by FLIP and caspase 8 signaling. Arthritis Rheum 2006;54:1473–81.
[19] Mauri DN, Ebner R, Montgomery RI, Kochel KD, Cheung TC, Yu GL, et al. LIGHT, a new member of the TNF superfamily, and lymphotoxin alpha are ligands for herpesvirus entry mediator. Immunity 1998;8:21–30.
[20] Crowe PD, VanArsdale TL, Walter BN, Ware CF, Hession C, Ehrenfels B, et al. A lymphotoxin-beta-specific receptor. Science 1994;264:707–10.
[21] Chen MC, Hwang MJ, Chou YC, Chen WH, Cheng G, Nakano H, et al. The role of apoptosis signal-regulating kinase 1 in lymphotoxin-beta receptor-mediated cell death. J Biol Chem 2003;278:16073–81.
[22] Tamada K, Shimozaki K, Chapoval AI, Zhu G, Sica G, Flies D, et al. Modulation of T-cell-mediated immunity in tumor and graft-versus-host disease models through the LIGHT co-stimulatory pathway. Nat Med 2000;6:283–9.
[23] Tumanov AV, Christiansen PA, Fu YX. The role of lymphotoxin receptor signaling in diseases. Curr Mol Med 2007;7:567–78.
[24] Wang J, Fu YX. Tumor necrosis factor family members and inflammatory bowel disease. Immunol Rev 2005;204:144–55.
[25] Lee WH, Kim SH, Lee Y, Lee BB, Kwon B, Song H, et al. Tumor necrosis factor receptor superfamily 14 is involved in atherogenesis by inducing proinflammatory cytokines and matrix metalloproteinases. Arterioscler Thromb Vasc Biol 2001;21:2004–10.
[26] Lo JC, Wang Y, Tumanov AV, Bamji M, Yao Z, Reardon CA, et al. Lymphotoxin beta receptor-dependent control of lipid homeostasis. Science 2007;316: 285–8.
[27] Edwards JR, Sun SG, Locklin R, Shipman CM, Adamopoulos IE, Athanasou NA, et al. LIGHT (TNFSF14), a novel mediator of bone resorption, is elevated in rheumatoid arthritis. Arthritis Rheum 2006;54:1451–62.
[28] Anders RA, Subudhi SK, Wang J, Pfeffer K, Fu YX. Contribution of the lymphotoxin beta receptor to liver regeneration. J Immunol 2005; 175:1295–300.
[29] Yang CR, Hsieh SL, Teng CM, Ho FM, Su WL, Lin WW. Soluble decoy receptor 3 induces angiogenesis by neutralization of TL1A, a cytokine belonging to tumor necrosis factor superfamily and exhibiting angiostatic action. Cancer Res 2004;64:1122–9.

[30] Chew LJ, Pan H, Yu J, Tian S, Huang WQ, Zhang JY, et al. A novel secreted splice variant of vascular endothelial cell growth inhibitor. FASEB J 2002;16:742–4.
[31] Bamias G, Kaltsa G, Siakavellas SI, Papaxoinis K, Zampeli E, Michopoulos S, et al. High intestinal and systemic levels of decoy receptor 3 (DcR3) and its ligand TL1A in active ulcerative colitis. Clin Immunol 2010;137:242–9.
[32] Bamias G, Siakavellas SI, Stamatelopoulos KS, Chryssochoou E, Papamichael C, Sfikakis PP. Circulating levels of TNF-like cytokine 1A (TL1A) and its decoy receptor 3 (DcR3) in rheumatoid arthritis. Clin Immunol 2008;129:249–55.
[33] Bamias G, Mishina M, Nyce M, Ross WG, Kollias G, Rivera-Nieves J, et al. Role of TL1A and its receptor DR3 in two models of chronic murine ileitis. Proc Natl Acad Sci USA 2006;103:8441–6.
[34] Yamazaki K, McGovern D, Ragoussis J, Paolucci M, Butler H, Jewell D, et al. Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. Hum Mol Genet 2005;14:3499–506.
[35] Green DR. Apoptosis. Death deceiver. Nature 1998;396:629–30.
[36] Roth W, Isenmann S, Nakamura M, Platten M, Wick W, Kleihues P, et al. Soluble decoy receptor 3 is expressed by malignant gliomas and suppresses CD95 ligand-induced apoptosis and chemotaxis. Cancer Res 2001;61:2759–65.
[37] Connolly K, Cho YH, Duan R, Fikes J, Gregorio T, LaFleur DW, et al. In vivo inhibition of Fas ligand-mediated killing by TR6, a Fas ligand decoy receptor. J Pharmacol Exp Ther 2001;298:25–33.
[38] Tsuji S, Hosotani R, Yonehara S, Masui T, Tulachan SS, Nakajima S, et al. Endogenous decoy receptor 3 blocks the growth inhibition signals mediated by Fas ligand in human pancreatic adenocarcinoma. Int J Cancer 2003;106:17–25.
[39] Chen PH, Yang CR. Decoy receptor 3 expression in AsPC-1 human pancreatic adenocarcinoma cells via the phosphatidylinositol 3-kinase-, Akt-, and NF-kappa B-dependent pathway. J Immunol 2008;181:8441–9.
[40] Zhang J, Salcedo TW, Wan X, Ullrich S, Hu B, Gregorio T, et al. Modulation of T-cell responses to alloantigens by TR6/DcR3. J Clin Invest 2001;107:1459–68.
[41] Han B, Wu J. DcR3 protects islet beta cells from apoptosis through modulating Adcyap1 and Bank1 expression. J Immunol 2009;183:8157–66.
[42] Hsu TL, Chang YC, Chen SJ, Liu YJ, Chiu AW, Chio CC, et al. Modulation of dendritic cell differentiation and maturation by decoy receptor 3. J Immunol 2002;168:4846–53.
[43] Hsu TL, Wu YY, Chang YC, Yang CY, Lai MZ, Su WB, et al. Attenuation of Th1 response in decoy receptor 3 transgenic mice. J Immunol 2005;175:5135–45.
[44] Chang YC, Hsu TL, Lin HH, Chio CC, Chiu AW, Chen NJ, et al. Modulation of macrophage differentiation and activation by decoy receptor 3. J Leukoc Biol 2004;75:486–94.
[45] Chang YC, Chen TC, Lee CT, Yang CY, Wang HW, Wang CC, et al. Epigenetic control of MHC class II expression in tumor-associated macrophages by decoy receptor 3. Blood 2008;111:5054–63.
[46] Hsu MJ, Lin WW, Tsao WC, Chang YC, Hsu TL, Chiu AW, et al. Enhanced adhesion of monocytes via reverse signaling triggered by decoy receptor 3. Exp Cell Res 2004;292:241–51.
[47] Yang CR, Hsieh SL, Ho FM, Lin WW. Decoy receptor 3 increases monocyte adhesion to endothelial cells via NF-kappa B-dependent up-regulation of intercellular adhesion molecule-1, VCAM-1, and IL-8 expression. J Immunol 2005;174:1647–56.
[48] Tateishi K, Miura Y, Hayashi S, Takahashi M, Kurosaka M. DcR3 protects THP-1 macrophages from apoptosis by increasing integrin alpha4. Biochem Biophys Res Commun 2009;389:593–8.
[49] Yang CR, Wang JH, Hsieh SL, Wang SM, Hsu TL, Lin WW. Decoy receptor 3 (DcR3) induces osteoclast formation from monocyte/macrophage lineage precursor cells. Cell Death Differ 2004;11(Suppl. 1):S97–107.
[50] Tang CH, Hsu TL, Lin WW, Lai MZ, Yang RS, Hsieh SL, et al. Attenuation of bone mass and increase of osteoclast formation in decoy receptor 3 transgenic mice. J Biol Chem 2007;282:2346–54.
[51] Wu YY, Chang YC, Hsu TL, Hsieh SL, Lai MZ. Sensitization of cells to TRAIL-induced apoptosis by decoy receptor 3. J Biol Chem 2004;279:44211–8.
[52] You RI, Chang YC, Chen PM, Wang WS, Hsu TL, Yang CY, et al. Apoptosis of dendritic cells induced by decoy receptor 3 (DcR3). Blood 2008;111:1480–8.
[53] Chang YC, Chan YH, Jackson DG, Hsieh SL. The glycosaminoglycan-binding domain of decoy receptor 3 is essential for induction of monocyte adhesion. J Immunol 2006;176:173–80.
[54] Li H, Zhang L, Lou H, Ding I, Kim S, Wang L, et al. Overexpression of decoy receptor 3 in precancerous lesions and adenocarcinoma of the esophagus. Am J Clin Pathol 2005;124:282–7.
[55] Takahama Y, Yamada Y, Emoto K, Fujimoto H, Takayama T, Ueno M, et al. The prognostic significance of overexpression of the decoy receptor for Fas ligand (DcR3) in patients with gastric carcinomas. Gastric Cancer 2002;5:61–8.
[56] Ibrahim SM, Ringel J, Schmidt C, Ringel B, Muller P, Koczan D, et al. Pancreatic adenocarcinoma cell lines show variable susceptibility to TRAIL-mediated cell death. Pancreas 2001;23:72–9.
[57] Arakawa Y, Tachibana O, Hasegawa M, Miyamori T, Yamashita J, Hayashi Y. Frequent gene amplification and overexpression of decoy receptor 3 in glioblastoma. Acta Neuropathol 2005;109:294–8.
[58] Macher-Goeppinger S, Aulmann S, Wagener N, Funke B, Tagscherer KE, Haferkamp A, et al. Decoy receptor 3 is a prognostic factor in renal cell cancer. Neoplasia 2008;10:1049–56.
[59] Connor JP, Felder M. Ascites from epithelial ovarian cancer contain high levels of functional decoy receptor 3 (DcR3) and is associated with platinum resistance. Gynecol Oncol 2008;111:330–5.
[60] Ohshima K, Haraoka S, Sugihara M, Suzumiya J, Kawasaki C, Kanda M, et al. Amplification and expression of a decoy receptor for fas ligand (DcR3) in virus (EBV or HTLV-I) associated lymphomas. Cancer Lett 2000;160:89–97.
[61] Chang PM, Chen PM, Hsieh SL, Tzeng CH, Liu JH, Chiou TJ, et al. Expression of a soluble decoy receptor 3 in patients with diffuse large B-cell lymphoma predicts clinical outcome. Int J Oncol 2008;33:549–54.
[62] Brunetti G, Oranger A, Mori G, Centonze M, Colaianni G, Rizzi R, et al. The formation of osteoclasts in multiple myeloma bone disease patients involves the secretion of soluble decoy receptor 3. Ann N Y Acad Sci 2010;1192:298–302.
[63] Tu HF, Liu CJ, Liu SY, Chen YP, Yu EH, Lin SC, et al. Serum decoy receptor 3 level: a predictive marker for nodal metastasis and survival among oral cavity cancer patients. Head Neck 2011;33:396–402.
[64] Chen G, Luo D. Expression of decoy receptor 3 in liver tissue microarrays. Natl Med J India 2008;21:275–8.
[65] Kim S, Kotoula V, Hytiroglou P, Zardavas D, Zhang L. Significance of increased expression of decoy receptor 3 in chronic liver disease. Dig Liver Dis 2009;41:591–8.
[66] Wu Y, Han B, Sheng H, Lin M, Moore PA, Zhang J, et al. Clinical significance of detecting elevated serum DcR3/TR6/M68 in malignant tumor patients. Int J Cancer 2003;105:724–32.
[67] Chen J, Zhang L, Kim S. Quantification and detection of DcR3, a decoy receptor in TNFR family. J Immunol Methods 2004;285:63–70.
[68] Simon I, Liu Y, Krall KL, Urban N, Wolfert RL, Kim NW, et al. Evaluation of the novel serum markers B7-H4, Spondin 2, and DcR3 for diagnosis and early detection of ovarian cancer. Gynecol Oncol 2007;106:112–8.
[69] Mild G, Bachmann F, Boulay JL, Glatz K, Laffer U, Lowy A, et al. DCR3 locus is a predictive marker for 5-fluorouracil-based adjuvant chemotherapy in colorectal cancer. Int J Cancer 2002;102:254–7.
[70] Shen HW, Gao SL, Wu YL, Peng SY. Overexpression of decoy receptor 3 in hepatocellular carcinoma and its association with resistance to Fas ligand-mediated apoptosis. World J Gastroenterol 2005;11:5926–30.
[71] Buffart TE, van Grieken NC, Tijssen M, Coffa J, Ylstra B, Grabsch HI, et al. High resolution analysis of DNA copy-number aberrations of chromosomes 8, 13, and 20 in gastric cancers. Virchows Arch 2009;455:213–23.
[72] Xiong G, Guo H, Wang K, Hu H, Wang D, Xu X, et al. Polymorphisms of decoy receptor 3 are associated with risk of esophageal squamous cell carcinoma in Chinese Han. Tumour Biol 2010;31:443–9.
[73] Douet-Guilbert N, Lai JL, Basinko A, Gueganic N, Andrieux J, Pollet B, et al. Fluorescence in situ hybridization characterization of ider(20q) in myelodysplastic syndrome. Br J Haematol 2008;143:716–20.
[74] Otsuki T, Tomokuni A, Sakaguchi H, Aikoh T, Matsuki T, Isozaki Y, et al. Overexpression of the decoy receptor 3 (DcR3) gene in peripheral blood mononuclear cells (PBMC) derived from silicosis patients. Clin Exp Immunol 2000;119:323–7.
[75] Kim S, McAuliffe WJ, Zaritskaya LS, Moore PA, Zhang L, Nardelli B. Selective induction of tumor necrosis receptor factor 6/decoy receptor 3 release by bacterial antigens in human monocytes and myeloid dendritic cells. Infect Immun 2004;72:89–93.
[76] Funke B, Autschbach F, Kim S, Lasitschka F, Strauch U, Rogler G, et al. Functional characterisation of decoy receptor 3 in Crohn's disease. Gut 2009;58:483–91.
[77] Lee CS, Hu CY, Tsai HF, Wu CS, Hsieh SL, Liu LC, et al. Elevated serum decoy receptor 3 with enhanced T cell activation in systemic lupus erythematosus. Clin Exp Immunol 2008;151:383–90.
[78] Chen CC, Yang YH, Lin YT, Hsieh SL, Chiang BL. Soluble decoy receptor 3: increased levels in atopic patients. J Allergy Clin Immunol 2004;114:195–7.
[79] Chen SJ, Wang YL, Kao JH, Wu SF, Lo WT, Wu CC, et al. Decoy receptor 3 ameliorates experimental autoimmune encephalomyelitis by directly counteracting local inflammation and downregulating Th17 cells. Mol Immunol 2009;47:567–74.
[80] Ka SM, Sytwu HK, Chang DM, Hsieh SL, Tsai PY, Chen A. Decoy receptor 3 ameliorates an autoimmune crescentic glomerulonephritis model in mice. J Am Soc Nephrol 2007;18:2473–85.
[81] Chen CY, Yang KY, Chen MY, Chen HY, Lin MT, Lee YC, et al. Decoy receptor 3 levels in peripheral blood predict outcomes of acute respiratory distress syndrome. Am J Respir Crit Care Med 2009;180:751–60.
[82] Ho CH, Hsu CF, Fong PF, Tai SK, Hsieh SL, Chen CJ. Epstein–Barr virus transcription activator Rta upregulates decoy receptor 3 expression by binding to its promoter. J Virol 2007;81:4837–47.
[83] Ho CH, Chen CL, Li WY, Chen CJ. Decoy receptor 3, upregulated by Epstein–Barr virus latent membrane protein 1, enhances nasopharyngeal carcinoma cell migration and invasion. Carcinogenesis 2009;30:1443–51.
[84] Kim S, Fotiadu A, Kotoula V. Increased expression of soluble decoy receptor 3 in acutely inflamed intestinal epithelia. Clin Immunol 2005;115:286–94.
[85] Fayad R, Brand MI, Stone D, Keshavarzian A, Qiao L. Apoptosis resistance in ulcerative colitis: high expression of decoy receptors by lamina propria T cells. Eur J Immunol 2006;36:2215–22.
[86] Maeda T, Hao C, Tron VA. Ultraviolet light (UV) regulation of the TNF family decoy receptors DcR2 and DcR3 in human keratinocytes. J Cutan Med Surg 2001;5:294–8.
[87] Bobe J, Goetz FW. A tumor necrosis factor decoy receptor homologue is up-regulated in the brook trout (*Salvelinus fontinalis*) ovary at the completion of ovulation. Biol Reprod 2000;62:420–6.
[88] Chen HF, Chen JS, Shun CT, Tsai YF, Ho HN. Decoy receptor 3 expression during the menstrual cycle and pregnancy, and regulation by sex steroids in endometrial cells in vitro. Hum Reprod 2009;24:1350–8.
[89] Sugimoto M, Kagawa N, Morita M, Kume S, Wongpanit K, Jin H, et al. Changes in the expression of decoy receptor 3 in granulosa cells during follicular atresia in porcine ovaries. J Reprod Dev 2010;56:467–74.

[90] Gill RM, Coleman NM, Hunt JS. Differential cellular expression of LIGHT and its receptors in early gestation human placentas. J Reprod Immunol 2007;74:1–6.
[91] Wu SF, Liu TM, Lin YC, Sytwu HK, Juan HF, Chen ST, et al. Immunomodulatory effect of decoy receptor 3 on the differentiation and function of bone marrow-derived dendritic cells in nonobese diabetic mice: from regulatory mechanism to clinical implication. J Leukoc Biol 2004;75:293–306.
[92] Wang YL, Chou FC, Sung HH, Fan PL, Hsueh CW, Lin WC, et al. Decoy receptor 3 protects non-obese diabetic mice from autoimmune diabetes by regulating dendritic cell maturation and function. Mol Immunol 2010;47:2552–62.
[93] Sung HH, Juang JH, Lin YC, Kuo CH, Hung JT, Chen A, et al. Transgenic expression of decoy receptor 3 protects islets from spontaneous and chemical-induced autoimmune destruction in nonobese diabetic mice. J Exp Med 2004;199:1143–51.
[94] Kawamoto K, Tanemura M, Nishida T, Fukuzawa M, Ito T, Matsuda H. Significant inhibition of human CD8(+) cytotoxic T lymphocyte-mediated xenocytotoxicity by overexpression of the human decoy Fas antigen. Transplantation 2006;81:789–96.
[95] Zhang J, Wang X, Fahmi H, Wojcik S, Fikes J, Yu Y, et al. Role of TL1A in the pathogenesis of rheumatoid arthritis. J Immunol 2009;183:5350–7.
[96] Bull MJ, Williams AS, Mecklenburgh Z, Calder CJ, Twohig JP, Elford C, et al. The Death Receptor 3–TNF-like protein 1A pathway drives adverse bone pathology in inflammatory arthritis. J Exp Med 2008;205:2457–64.
[97] Ishida S, Yamane S, Ochi T, Nakano S, Mori T, Juji T, et al. LIGHT induces cell proliferation and inflammatory responses of rheumatoid arthritis synovial fibroblasts via lymphotoxin beta receptor. J Rheumatol 2008;35:960–8.
[98] Mueller AM, Pedre X, Killian S, David M, Steinbrecher A. The decoy receptor 3 (DcR3, TNFRSF6B) suppresses Th17 immune responses and is abundant in human cerebrospinal fluid. J Neuroimmunol 2009;209:57–64.
[99] Kugathasan S, Baldassano RN, Bradfield JP, Sleiman PM, Imielinski M, Guthery SL, et al. Loci on 20q13 and 21q22 are associated with pediatric-onset inflammatory bowel disease. Nat Genet 2008;40:1211–5.
[100] Wroblewski VJ, McCloud C, Davis K, Manetta J, Micanovic R, Witcher DR. Pharmacokinetics, metabolic stability, and subcutaneous bioavailability of a genetically engineered analog of DcR3, FLINT [DcR3(R218Q)], in cynomolgus monkeys and mice. Drug Metab Dispos 2003;31:502–7.