

Immunobiology of Human NKG2D and Its Ligands

S. González · V. Groh · T. Spies (✉)

Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N.,
Seattle, WA 98109, USA
tspies@fhcrc.org

1	Introduction	122
2	Structure and Regulation of MIC	123
3	Tumor-Associated and Pathogen-Induced Expression of MIC	125
4	The ULBP Family of NKG2D Ligands	126
5	NKG2D and Its Physical Interactions with MIC and ULBP	127
6	Activating and Costimulatory Functions of NKG2D	129
7	Viral and Tumor Immune Evasion	130
8	Role of MIC-NKG2D in Autoimmune Diseases	131
9	Recognition of MIC by Intraepithelial $\gamma\delta$ T Cells	132
10	Polymorphism of MIC and Disease Associations	133
	References	134

Abstract The NKG2D-DAP10 receptor complex activates natural killer (NK) cells and costimulates effector T cell subsets on engagement of ligands that can be conditionally expressed under physiologically harmful conditions such as microbial infections and malignancies. These characteristics have given rise to the widely embraced concept of immunorecognition of “induced or damaged self,” complementing the “missing self” paradigm that is represented by MHC class I allotypes and their interactions with inhibitory receptors on NK cells. However, this notion may only be partially sustainable, as various patterns of constitutive tissue distributions have become apparent among members of one NKG2D ligand family. This review summarizes the biological properties of NKG2D and its ligands and discusses the interactions and regulation of these molecules with emphasis on their significance in microbial infections, tumor immunology, and autoimmune disease.

1 Introduction

When NK cells engage target cells, the aggregate signals from inhibitory and activating receptors are integrated into balances that control their effector functions. Among these natural killer receptors (NKR) are inhibitory or activating isoforms of the killer cell Ig-like receptors (KIR) and the inhibitory leukocyte Ig-like receptor (LIR)-1, which bind to HLA-A, -B, or -C alleles, and the C-type lectin-like inhibitory CD94-NKG2A and activating CD94-NKG2C heterodimers, which interact with HLA-E (Lee et al. 1998; Long 1999). Inhibitory receptors have higher ligand affinities than their activating isoforms and thus convey dominant-negative signals (Lanier 2001). They have cytoplasmic immunoreceptor tyrosine-based inhibition motifs (ITIM), which function by recruitment of SHP-1 tyrosine phosphatases (Ravetch and Lanier 2000). Activating KIR isoforms, which lack ITIM, and the CD94-NKG2C receptor associate with an adaptor protein, DAP12, which has a cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM) and signals similar to the CD3 ζ and Fc ϵ R1 γ chains, by recruitment of Syk or ZAP-70 tyrosine kinases (Lanier 2001). Additional activating receptors include members of the LIR family and the Nkp30, Nkp44, and Nkp46 proteins, which are also referred to as natural cytotoxicity receptors (Borges et al. 1997; Pessino et al. 1998; Cantoni et al. 1999; Pende et al. 1999). However, the significance and ligand interactions of these molecules are less well defined. In their interactions with inhibitory NKR, MHC class I molecules function as passports certifying the integrity of cells. Because their expression is often impaired by viral infections and tumorigenesis, insufficient engagement of inhibitory NKR results in target cell susceptibility to lysis by NK cells. Because NK cells express variable arrays of inhibitory NKR with different ligand specificities, they are enabled to detect loss of individual MHC class I alleles. Additional expression of KIR or CD94-NKG2A on T cells after persistent antigen-driven stimulation results in increased T cell antigen receptor (TCR)-dependent activation thresholds and T cell anergy, thus effecting downmodulation of effector responses in chronic infections and malignancies, which may safeguard against autoimmune reactions (Noppen et al. 1998; Moser et al. 2002).

Whereas most NKR bind MHC class I molecules that are ubiquitously expressed, the activating NKG2D receptor interacts with distant relatives of MHC class I, some of which are inducibly expressed (Lanier 2001; Raulet 2003). Among these, the prototype ligands are the closely related MICA and MICB, which are regulated by cellular stress. The tissue distribution of these proteins is restricted to intestinal epithelium, but they can be induced by some microbial infections and are frequently associated with epithelial tumors of

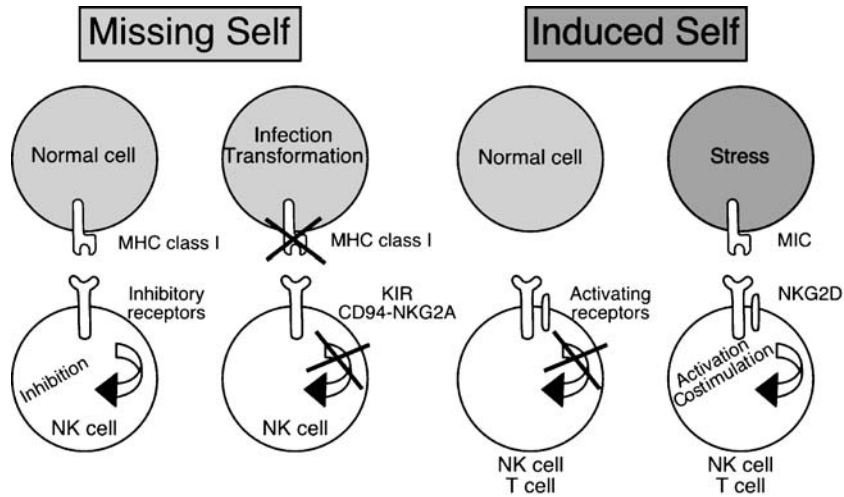


Fig. 1 Regulation of NK cell responses by inhibitory NKR interacting with MHC class I molecules and by NKG2D on engagement by its inducible MIC ligands

diverse tissue origins. NKG2D is encoded by a gene linked to the NKG2 receptor family, although it bears no sequence resemblance. Engagement of NKG2D by MIC potentially activates NK cell functions and costimulates effector T cell responses. Thus MIC deliver an “induced or damaged self” signal that is coupled to cellular changes caused by microbes or malignant cell growth, thereby alerting the immune system to harmful conditions (Fig. 1) (Raulet 2003). This system bears superficial similarity to the recognition of pathogen-associated molecular patterns (PAMP) by the family of Toll-like receptors (TLR). Additional NKG2D ligands are represented by a family of at least five ULBP proteins. However, the tissue distribution, regulation, and significance of these molecules are not well defined.

2 Structure and Regulation of MIC

MICA and MICB are encoded by genes near *HLA-B*, and homologous dysfunctional pseudogene sequences are located in the vicinities of *HLA-A*, *-E*, and *-G* (Bahram et al. 1994; Bahram and Spies, 1996). The 43-kD MICA and MICB core polypeptides share 84% amino acid sequence homology and are distinctively related to mammalian MHC class I chains as compared to the class I-like CD1 and Fc-receptor molecules encoded elsewhere in the genome.

The MIC protein sequences are about equidistant from all mammalian MHC class I chains, sharing about 30% identical amino acid residues throughout the aligned extracellular $\alpha 1$, $\alpha 2$, and $\alpha 3$ domains. Further characteristic of these molecules are seven or eight *N*-linked glycosylation sites, unique transmembrane and cytoplasmic tail sequences, three extra cysteine residues in the $\alpha 1$ and $\alpha 3$ domains, and the absence of all of the amino acid residues involved in binding of CD8. Sequences orthologous to MIC are conserved in the genomes of all mammalian species examined, with the exception of rodents (Bahram et al. 1994). MIC genes are functionally expressed in diverse nonhuman primates and presumably most other mammals (Steinle et al. 1998). MIC proteins are not associated with β_2 -microglobulin (β_2m), and their transport is independent of the peptide processing machinery that is required for the assembly of peptide antigen-presenting MHC class I molecules (Groh et al. 1996). Moreover, gel-filtration chromatography of acid-treated MICA isolated from cells after labeling with tritiated amino acids provided no evidence for bound peptides (Groh et al. 1998). These characteristics are reflected in the crystal structure of MICA, which shows a dramatically altered class I fold with only a shallow remnant of a peptide-binding groove and restructured $\alpha 1\alpha 2$ platform and $\alpha 3$ domain interfaces that preclude binding of β_2m (Li et al. 1999).

Unlike that of the ubiquitous MHC class I molecules, the tissue distribution of MIC is normally restricted to variable areas of the intestinal epithelium, with limited evidence for surface expression (Groh et al. 1996). In cultured polarized epithelial cells, the proteins are sorted to basolateral epithelial membranes by an active process that is determined by two adjacent hydrophobic amino acids, leucine and valine, at the membrane-proximal ends of their cytoplasmic tails (Suemizu et al. 2002). The immunobiology and regulatory mechanisms underlying the expression of MIC in intestinal epithelium are poorly understood. Among tissue culture cell lines, expression of MIC is mostly limited to fibroblast and epithelial cells and is not inducible by interferons. Importantly, however, MIC can be heat shock-induced similar to *heat shock protein 70* (*hsp70*) genes, presumably because of the presence of a highly conserved heat shock response element (HSE) in the 5'-flanking regions of the corresponding genes (Groh et al. 1996, 1998). Data from electrophoretic mobility shift assays (EMSA) and in vivo genomic footprinting (IVGF) have confirmed that these motifs specifically bind heat shock factor-1 (HSF-1), which is the dominant transcription factor controlling the expression of *hsp70* genes (D. Suciú and T. Spies, unpublished data; Morimoto et al. 1992). In accord with the mode of *hsp70* regulation, MIC are expressed in significant amounts on rapidly proliferating epithelial cell lines but are scarce on quiescent cells grown to high confluence. Under this condition, exposure

to heat shock results in a maximal 10-fold amplification of MIC mRNAs and surface proteins (Groh et al. 1998). Hence, *MIC* can be regarded as cell stress response genes.

3 Tumor-Associated and Pathogen-Induced Expression of MIC

MIC are frequently expressed in many, but not all, lung, breast, kidney, ovarian, prostate, gastric, and colon carcinomas and melanomas (Groh et al. 1999; Vetter et al. 2002). There are high degrees of variability in the proportions of tumor cells that are positive for MIC. The physiological reasons are unknown, but they could be related to local stress-inducing conditions such as tumor cell proliferation, hypoxia, and hyperglycemia. Oxidative stress has been shown to increase *MIC* gene expression in a colon carcinoma cell line (Yamamoto et al. 2001). Modest MIC expression has also been reported in some hematopoietic malignancies (AML, ALL, and CML), perhaps most significantly in multiple myeloma (Salih et al. 2003).

MIC are strongly induced in fibroblast and endothelial cells by cytomegalovirus (CMV) and by *Mycobacterium tuberculosis* infection in dendritic and epithelial cells (Das et al. 2001; Groh et al. 2001). MICB is induced by Sendai and influenza A virus infection in macrophages (Siren et al. 2004). Skin lesions of lepromatous lepra patients are marked by large amounts of MIC (V. Groh and T. Spies, unpublished data). Intestinal epithelial expression of MIC may be inducible by bacteria, because adhesion of diarrheagenic *Escherichia coli* strains to the intestinal epithelial Caco-2 cell line induces a rapid increase of MICA (Tieng et al. 2002). This effect has been related to an interaction of the bacterial AfaE-III adhesin with the cellular CD55 receptor, a glycosylphosphatidylinositol (GPI)-anchored protein that is expressed on most human cells and inhibits complement C3b deposition. However, how this interaction might result in MIC induction has remained unclear. So far, these are the only examples establishing a connection between infectious agents and MIC expression, suggesting that the scope of a more universal function of MIC in infectious diseases remains to be fully explored. This is of particular significance because immune response systems have primarily evolved by pathogen-driven selection. In the mouse, members of the retinoic acid early inducible-1 (RAE-1) family of NKG2D ligands are induced in macrophages on stimulation of TLR by microbial products (Hamerman et al. 2004). However, similar observations have not been reported for the human MIC or ULBP ligands so far.

4 The ULBP Family of NKG2D Ligands

Binding studies utilizing recombinant IgG Fc region fusion proteins led to the discovery that the CMV UL16 transmembrane glycoprotein, which was of unknown function, specifically interacts with cell surface proteins that were termed ULBP1 and ULBP2 (Cosman et al. 2001). Three additional ULBP sequences were identified by sequence homology (Fig. 2) (Chalupny et al. 2003; Bacon et al. 2004). ULBP3 and -4 and MICA do not interact with UL16, but MICB does. ULBP share no direct sequence relationship with MIC and are encoded outside the MHC on chromosome 6q25. As with MIC, ULBP are distant members of the MHC class I family. All ULBP lack the membrane proximal $\alpha 3$ domain. ULBP1–3, and ULBP4 and -5 have GPI membrane anchors and transmembrane regions, respectively. None of these molecules is associated with β_2m or peptide ligands. The $\alpha 1\alpha 2$ domains of ULBP share about 50%–60% identical amino acids and are equidistant from those of MHC class I and MIC, with about 25% sequence homology. ULBP1–3 are moderately induced by CMV infection (Welte et al. 2003). Although ULBP mRNAs are quite ubiquitously expressed, little is known regarding the tissue distribution and regulation of the encoded proteins. Preliminary data indicate diverse constitutive expression patterns in epithelia, endothelia, and antigen-presenting cells (V. Groh and T. Spies, unpublished data). This may oppose the “induced or damaged self” hypothesis and warrants further investigation (Fig. 1). In outer cell membranes, the GPI-anchored ULBP are clustered in lipid raft microdomains, which may serve to create enhanced avidity because at least mouse GPI-anchored NKG2D ligands have lower receptor affinities than those with transmembrane domains (Eleme et al. 2004). Indeed, these ULBP and MICA, which is S-acylated, accumulate at NK cell immune synapses. With polarized epithelial cells, lipid rafts form preferentially at apical membrane surfaces. Disruption of epithelial tight junctions, for example, by processes of infection or tumorigenesis, could allow GPI-anchored ULBP to diffuse toward basolateral surfaces where they would become exposed to NKG2D-bearing lymphocytes. Currently, there are no experimentally validated models explaining the significance of the conservation of the two families of highly diversified NKG2D ligands. However, it seems probable that these have been selected to serve as indicators of diverse pathological conditions in different tissue environments by adoption of distinct strategies, namely, cell stress response-coupled transcriptional induction of MIC and, perhaps, induction of immunological visibility of GPI-anchored ULBP by alterations of cellular and tissue integrities.

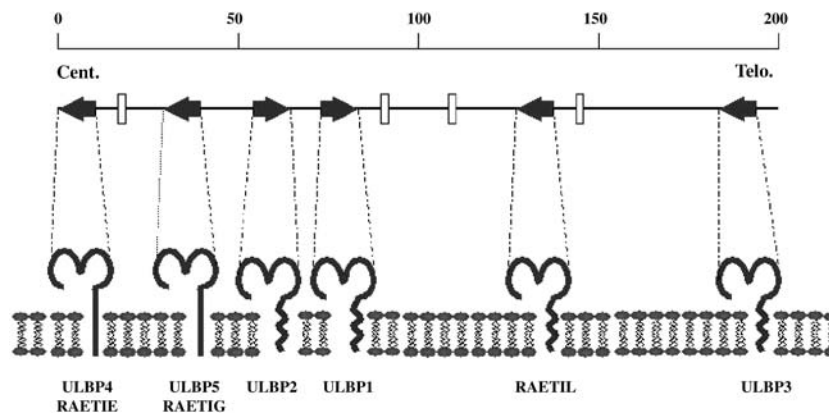


Fig. 2 Schematic depiction of the genetic organization of the *ULBP* gene family of NKG2D ligands. *Arrows* indicate transcriptional orientation of genes on 6q24.2–q25.3. RAET1L encodes a potentially functional gene but has not been further characterized

5 NKG2D and Its Physical Interactions with MIC and ULBP

Formerly an orphan receptor with unknown function (Houchins et al. 1991), NKG2D was first identified as a receptor for MICA and MICB and subsequently for the ULBP ligands (Bauer et al. 1999; Cosman et al. 2001; Chalupny et al. 2003; Bacon et al. 2004). Recombinant NKG2D binds firmly to transfectants expressing its ligands, as do recombinant ligands to lymphocyte subsets expressing NKG2D (Steinle et al. 2001). Analysis by size-exclusion chromatography showed that NKG2D homodimers form stable complexes with monomeric MICA in solution, thus demonstrating that no other components are required to facilitate this interaction. Glycosylation of NKG2D or MICA is not essential but enhances complex formation (Steinle et al. 2001). NKG2D is a type II membrane glycoprotein of 42 kD with a core polypeptide of 28 kD that is expressed on most NK cells, $\gamma\delta$ T cells, CD8 $\alpha\beta$ T cells, and a subset of NK T cells and thus is the most broadly distributed NKR known (Bauer et al. 1999). It shares no direct relationship with other NKG2 proteins and is not associated with CD94. Instead, NKG2D pairs via interactions between oppositely charged transmembrane amino acids with the DAP10 adaptor protein, which signals similar to CD28 by activation of the p85 subunit of phosphatidylinositol 3-kinase (PI3-K) on tyrosine phosphorylation of a YXXM motif in its cytoplasmic domain (Fig. 3) (Wu et al. 1999). In mouse NK cells, an alternatively spliced, shortened form of NKG2D can associate with DAP12 (Diefenbach et al. 2002); however, there is no corresponding vari-

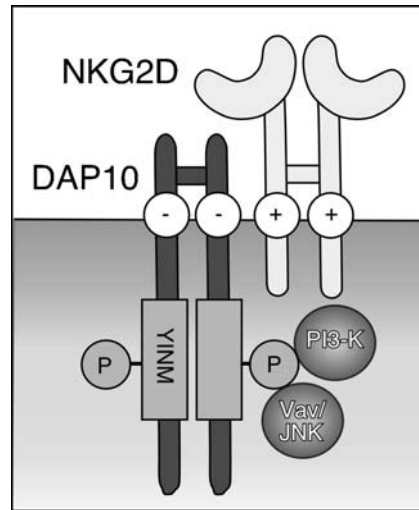


Fig. 3 Composition of the NKG2D-DAP10 receptor complex and signaling via PI3-K-dependent and -independent pathways

ant of NKG2D and alternative association with DAP12 in humans (Rosen et al. 2004).

In a complex crystal structure, the saddle-shaped NKG2D homodimer sits astride the $\alpha 1\alpha 2$ platform domain of MICA, with each NKG2D monomer contacting either the $\alpha 1$ or the $\alpha 2$ subdomain (Li et al. 2001). The footprint of NKG2D on MICA largely overlaps that of $\alpha\beta$ TCR on MHC class I-peptide ligands, despite the lack of structural similarity between the Ig-like domains of TCR and the C-type lectin-like domains of NKG2D. The rigidity of the interaction of NKG2D with MICA and diverse other ligands involves an investment of the majority of the interaction energy in two binding site core tyrosines (at positions 152 and 199) that are able to make distinct, dominant interactions at each interface in the absence of conformational plasticity. This is distinct from “induced-fit” or “preexisting equilibrium” mechanisms that can be involved in $\alpha\beta$ TCR- and antibody-mediated recognition (McFarland and Strong 2003). With the additional information obtained from a NKG2D-ULBP3 complex structure (Radaev et al. 2001), the perplexing ability of NKG2D to interact with highly diverse ligands has been suggested to involve common interfaces and distinct but overlapping sets of hydrogen bonds, hydrophobic interactions, and salt bridges, thus permitting conservation of general shape complementarities and binding energies (Radaev et al. 2002).

6 Activating and Costimulatory Functions of NKG2D

Engagement of NKG2D by any of its ligands on transfectants or by MIC on diverse epithelial tumor cells activates NK cells in the presence of inhibitory NKR and their respective MHC class I ligands (Bauer et al. 1999). Moreover, ectopic expression by transfection of murine RAE-1 ligands causes NKG2D-dependent rejection of tumor cells by NK cells and primed cytotoxic T cells in syngeneic mice, thus reinforcing the potential significance of human MIC-NKG2D in innate and adaptive immune responses against tumors (Cerwenka et al. 2001; Diefenbach et al. 2001). Whereas NKG2D has the capacity to trigger NK cells, it costimulates CD8 $\alpha\beta$ T cells and $\gamma\delta$ T cells (Das et al. 2001; Groh et al. 2001; Roberts et al. 2001). Cytotoxicity assays with CMV or melanoma antigen-specific CD8 $\alpha\beta$ T cells have shown that engagement of NKG2D by MIC strongly augments T cell responses under conditions of suboptimal MHC-peptide antigen stimulation of TCR (Groh et al. 2001, 2002; Vetter et al. 2002). Induced expression of MIC can thus overcome interference of viral gene products with antigen processing and presentation and the downmodulation of MHC class I that is frequently associated with tumors. However, even at optimal stimulation of TCR, NKG2D potently costimulates the production of cytokines, including interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α), and interleukin-2 (IL-2) and IL-4, and T cell proliferation (Groh et al. 2001). Similarly, NKG2D costimulates $V\gamma_2/V\delta_2$ T cells, which recognize bacterial and mycobacterial soluble organic phosphate and alkamine antigens in a non-MHC-restricted manner. Infection by *Mycobacterium tuberculosis* induces expression of MIC on dendritic and epithelial cells, resulting in NKG2D-mediated costimulation of $V\gamma_2/V\delta_2$ T cell cytotoxicity, proliferation, and release of IFN- γ and IL-2 (Das et al. 2001). The induction of MIC and function of NKG2D thus offer an explanation for why these T cells expand dramatically only during microbial infections although they are capable of recognizing antigen-related moieties that are abundant in uninfected individuals.

NKG2D can also trigger T cells in a TCR-independent manner. Normal freshly isolated intestinal intraepithelial lymphocytes (IEL) exhibit markedly diminished expression of NKG2D, which may be downmodulated to prevent chronic T cell stimulation and autoreactive bystander T cell activation. High levels of NKG2D can be induced by IL-15 (Roberts et al. 2001), which is produced by intestinal epithelial cells on external stimuli and infection. In patients with active celiac disease, however, NKG2D is strongly expressed because of high local levels of IL-15 and MIC is upregulated in intestinal epithelial cells (Hue et al. 2004; Meresse et al. 2004). Under these conditions,

freshly isolated intraepithelial CD8 $\alpha\beta$ T cells lyse intestinal epithelial cell lines independent of TCR engagement. NKG2D-DAP10 signaling involves both PI3-K-dependent and -independent (Vav/JNK) pathways (Fig. 3). This activation mode was also observed with normal peripheral blood effector stage CD8 T cells cultured as lymphokine-activated killer (LAK) cells in the presence of high doses of IL-15 (Meresse et al. 2004).

7 Viral and Tumor Immune Evasion

The existence of numerous specific interactions between viral proteins and molecules of the immune system reflects the intense evolutionary pressure imposed on host-pathogen relationships. Usually, these constitute a balance between viral escape from immune control and the host defense limiting virus spread and resultant disease. Human CMV persists lifelong in a latent state with asymptomatic episodes of virus shedding. Only in immunocompromised individuals does virus reactivation result in severe disease manifestations. To maintain long-term persistence in infected hosts, CMV interferes with several stages of antigen processing and presentation by MHC class I molecules, thus compromising the ability of CD8 $\alpha\beta$ T cells to eliminate infected cells. The CMV US6 membrane protein impairs the function of TAP, which delivers peptides into the endoplasmic reticulum (ER) for binding to MHC class I molecules. The US3 protein retains class I molecules in the ER, and US2 and US11 redirect nascent class I chains back into the cytosol, where they are degraded (Ploegh 1998). Perhaps not surprisingly, CMV also has the capacity to obstruct the function of NKG2D ligands: UL16 retains ULBP1, ULBP2, and MICB intracellularly via localization to or retrieval from the *trans*-Golgi network, thus abrogating surface expression (Dunn et al. 2003; Welte et al. 2003; Wu et al. 2003). This retention is mediated by a tyrosine-based motif in the cytoplasmic tail sequence of UL16. Deletion of this motif restores surface expression of the NKG2D ligands, whereas UL16 is redirected to endosomal compartments (Wu et al. 2003). However, this mechanism of CMV immune evasion is bypassed by ULBP3 and MICA, which are not bound by UL16.

In patients with epithelial tumors that are positive for MIC, large proportions of tumor-infiltrating lymphocytes (TIL) have low levels of NKG2D as a result of ligand-induced endocytosis and at least partial lysosomal degradation (Groh et al. 2002). In addition, NKG2D is systemically diminished on matched peripheral blood T cells and NK cells. This deficiency is associated with circulating tumor-derived soluble MICA and presumably MICB, which

cause the downregulation of NKG2D. As a result, the responsiveness of tumor antigen-specific effector T cells and NK cells is severely impaired (Groh et al. 2002; Doubrovina et al. 2003). Thus tumor shedding of MIC, which is probably due to the activity of metalloproteinases (Salih et al. 2002), may promote tumor immune evasion. Moreover, NKG2D is also downmodulated, albeit less substantially, by transforming growth factor β 1 (TGF- β 1) (Castriconi et al. 2003; Lee et al. 2004).

8

Role of MIC-NKG2D in Autoimmune Diseases

Because ligand binding unconditionally triggers NKG2D without counterbalance by a known antagonist, its dysregulation together with anomalous expression of MIC in local tissue sites may promote autoreactive T cell stimulation. Indeed, recent evidence indicates that MIC-NKG2D may play important roles in the pathogenesis of several autoimmune diseases. In rheumatoid arthritis (RA), the severity of autoimmune and inflammatory joint disease correlates with large numbers of CD4⁺CD28⁻ T cells, which are scarce in healthy individuals. Large proportions of these T cells aberrantly express NKG2D, which is absent from almost all normal CD4 T cells. NKG2D is induced by TNF- α and IL-15, which are present in RA synovia and RA patient sera. RA synoviocytes aberrantly express MIC, presumably because of pannus invasion, and thus stimulate autologous CD4⁺CD28⁻ T cell cytokine production and proliferation (Groh et al. 2003). As with cancer patients, RA serum contains substantial amounts of soluble MIC, which fails to downmodulate NKG2D because of the opposing activity of TNF- α and IL-15. Thus, by causing autoreactive T cell stimulation, MIC-NKG2D may promote the self-perpetuating pathology in RA (Groh et al. 2003). CD4⁺CD28⁻ T cells are also expanded in other autoimmune diseases and chronic inflammatory conditions, including multiple sclerosis, Wegener granulomatosis, ankylosing spondylitis, atherosclerotic coronary artery disease, and inflammatory bowel disease, suggesting the possibility of an involvement of MIC-NKG2D. In active celiac disease, upregulation of MIC on enterocytes by gliadin or its p31–49 peptide triggers NKG2D-dependent activation of IEL, resulting in cytotoxicity against epithelial targets and enhanced TCR-dependent CD8 T cell responses (Hue et al. 2004; Meresse et al. 2004). IL-15-mediated induction of NKG2D and resultant TCR-independent T cell activation may also contribute to villous atrophy (Meresse et al. 2004).

9 Recognition of MIC by Intraepithelial $\gamma\delta$ T Cells

Although $V\gamma_2/V\delta_2$ T cells predominate in the circulation, a small subset of $\gamma\delta$ T cells defined by the expression of $V\delta_1$ is enriched in intestinal epithelium and other epithelial sites. Some of these T cells recognize CD1c, a member of the CD1 family of lipid antigen-presenting molecules. In addition, numerous $V\delta_1$ $\gamma\delta$ T cell lines and clones with substantial sequence diversity in the rearranged γ (V-N-J) and δ (V-NDN-J) chains, including variability in nontemplated (N) sequences and numbers of D segments, respond against diverse target cells expressing MICA or MICB (Groh et al. 1998, 1999). These responses are dependent on triggering of TCR and NKG2D, posing the conundrum of whether the $\gamma\delta$ TCR recognize MIC or an unidentified surface moiety. This was resolved by demonstration of MICA tetramer binding to various $V\delta_1$ $\gamma\delta$ TCR expressed on transfectants of a T cell line selected for lack of NKG2D (Wu et al. 2002). Tetramer binding was restricted to TCR derived from responder T cell clones classified as reactive against a broad range of MIC-expressing epithelial tumor and transfectant target cells and was abrogated when TCR were composed of mismatched γ and δ chains. These observations, and the inability of $V\delta_1$ $\gamma\delta$ T cells to respond against target cells expressing ULBP ligands of NKG2D, support the model that MIC delivers both the TCR-dependent signal 1 and the NKG2D-dependent costimulatory signal 2 for activation of a subset of $V\delta_1$ $\gamma\delta$ T cells (Wu et al. 2002). This dual function has precedent in the manifold interactions of MHC class I molecules with $\alpha\beta$ TCR, the CD8 coreceptor, KIR, and LIR. The $\gamma\delta$ TCR-mediated recognition of MIC validates an earlier hypothesis derived from studies of mouse dendritic epidermal T cells, that intraepithelial $\gamma\delta$ T cells may recognize stress-inducible self antigens (Havran et al. 1991). At least in humans, this is corroborated by the colocalization of intraepithelial $V\delta_1$ $\gamma\delta$ T cells and MIC in tissue environments that include the intestinal mucosa, sites of viral infection, and epithelial tumors. A potentially important role of $V\delta_1$ $\gamma\delta$ T cells in antitumor immune responses is supported by experiments showing that mice lacking $\gamma\delta$ T cells are highly susceptible to carcinogen-induced skin malignancies. Exposure to carcinogens induces skin expression of RAE-1 ligands, which stimulate NKG2D-dependent $\gamma\delta$ T cell cytotoxicity (Girardi et al. 2001). The requirement of NKG2D for activation of the human $V\delta_1$ $\gamma\delta$ T cells may be due to suboptimal TCR stimulation by MIC. This may not be the case with $V\delta_1$ $\gamma\delta$ T cells specific for CD1c, which respond against target cells lacking demonstrated expression of NKG2D ligands (Spada et al. 2000).

10 Polymorphism of MIC and Disease Associations

MICA and to a lesser extent MICB are polymorphic, comprising more than 50 and about 15 amino acid substitutions in their extracellular $\alpha 1\alpha 2\alpha 3$ domains, respectively (Stephens 2001). Unlike MHC class I alleles, all these substitutions are only biallelic and appear randomly distributed. Little is known regarding the functional significance of this allelic variation; however, many substitutions are not conservative, suggesting evolutionary selection instead of random fixation. Mapping onto the MICA crystal structure suggests that some variant amino acid positions may affect interactions with NKG2D whereas most are distant from the NKG2D binding platform or buried inside the folded polypeptide. Analysis of the binding strength of soluble recombinant NKG2D to transfectants expressing five of the most frequent MICA alleles has revealed substantial variations in binding affinities in the range of 10- to 50-fold. These differences are associated with a single amino acid substitution at position 129, methionine or valine, which determines strong (MICA*01 and *07) and weak binding (MICA*04, *08 and *016) alleles, respectively (Steinle et al. 2001). This polymorphism may affect thresholds of NK and T cell activation. In the crystal structure of MICA*01, position 129 is located in the $\beta 4$ strand of the β -pleated sheet in the $\alpha 2$ domain. Because the side chain of methionine is partially buried and forms hydrophobic interactions with glutamine 136, alanine 139, and methionine 140 in the first $\alpha 2$ helical stretch, its substitution by valine likely affects NKG2D binding indirectly by a conformational change.

Extensive sequence diversity occurs within the MICA transmembrane region, mainly in the number of polyalanine repeats associated with different alleles (Stephens 2001). The MICA*08 allele, which has the highest frequency in Caucasians and Oriental populations, has a premature stop codon resulting in loss of part of the transmembrane region and the cytoplasmic tail. This protein is membrane anchored but fails to be properly sorted in polarized epithelial cells (Suemizu et al. 2002). Another defective allele is MICA*010, which has a single proline for arginine substitution at position 6 in the first β -strand of the $\alpha 1$ domain. This change blocks a β -sheet hydrogen bond with the histidine carbonyl at position 27 on the $\beta 2$ -strand and is incompatible with β -sheet secondary structure, thus interfering with a stable protein fold (Li et al. 2000). Of particular interest is a MIC-null haplotype associated with HLA-B*4801 that is relatively common among the Japanese and very frequent (56.5%) within an Amerindian community in Paraguay (Ota et al. 2000; Rusomando et al. 2002). In this haplotype, the entire *MICA* gene is within a 100-(kb deletion and *MICB* has a stop codon in exon 3 encoding the $\alpha 2$ do-

main. Because individuals homozygous for this haplotype have no discernible immunological deficiency, and significant common disease histories are not apparent, these observations have led to the conclusion that MIC function may not be essential or part of a redundant system. However, a more compelling explanation may eventually emerge, perhaps that loss of MIC expression may confer a selective advantage under certain environmental conditions.

Numerous studies have investigated relationships between MICA alleles and susceptibility to diseases that are associated with the closely linked *HLA-B* and *-C* genes, including ankylosing spondylitis, psoriasis vulgaris, psoriatic arthritis, and Behçet disease (Stephens 2001). However, positive associations are likely secondary because of strong linkage disequilibrium between *MICA* and the two MHC class I genes nearby and have not been confirmed by analyses of different HLA haplotypes in diverse ethnic groups. *MICA* has also been associated with MHC class II-linked diseases such as insulin-dependent diabetes mellitus (IDDM), Addison disease, sclerosing cholangitis, and celiac disease. However, as yet there is no evidence of a primary genetic association of *MICA* or *MICB* with any disease, and the functional significance of most of the allelic variation of these genes has remained unclear.

As with MHC class I molecules, a direct consequence of *MICA* polymorphism is the occurrence of autoantibodies in patients with irreversible rejection of allogeneic kidney and pancreas transplants. These show epithelial expression of MIC, which is not seen with normal organs or nonrejected transplants (Hankey et al. 2002; Sumitran-Holgersson et al. 2002). Thus MIC may contribute to allograft rejection, suggesting that matching of donor and recipients may improve clinical outcomes.

Acknowledgements S.G. was supported by the Spanish Fondo de Investigaciones Sanitarias (PI030067). Work from the authors' laboratory was supported by National Institutes of Health Grants AI-30581 and AI-52319.

References

- Bacon L, Eagle RA, Meyer M, Easom N, Young NT, Trowsdale J (2004) Two human ULBP/RAET1 molecules with transmembrane regions are ligands for NKG2D. *J Immunol* 173:1078–1084
- Bahram S, Bresnahan M, Geraghty DE, Spies T (1994) A second lineage of mammalian major histocompatibility complex class I genes. *Proc Natl Acad Sci USA* 91:6259–6263
- Bahram S, Spies T (1996) Nucleotide sequence of a human MHC class I MICB cDNA. *Immunogenetics* 43:230–233
- Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T (1999) Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* 285:727–729

- Borges L, Hsu M-L, Fanger N, Kubin M, Cosman D (1997) A family of human lymphoid and myeloid Ig-like receptors, some of which bind to MHC class I molecules. *J Immunol* 159:5192–5196
- Cantoni C, Bottino C, Vitale M, Pessino A, Augugliaro R, Malaspina A, Parolini S, Moretta L, Moretta A, Biassoni R (1999) NKp44, a triggering receptor involved in tumor cell lysis by activated human natural killer cells, is a novel member of the immunoglobulin superfamily. *J Exp Med* 189:787–796
- Castriconi R, Cantoni C, Della Chiesa M, Vitale M, Marcenaro E, Conte R, Biassoni R, Bottino C, Moretta L, Moretta A (2003) Transforming growth factor β 1 inhibits expression of NKp30 and NKG2D receptors: consequences for the NK-mediated killing of dendritic cells. *Proc Natl Acad Sci USA* 100:4120–4125
- Cerwenka A, Baron JL, Lanier LL (2001) Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor in vivo. *Proc Natl Acad Sci USA* 98:11521–11526
- Chalupny NJ, Sutherland CL, Lawrence WA, Rein-Weston A, Cosman D (2003) ULBP4 is a novel ligand for human NKG2D. *Biochem Biophys Res Commun* 305:129–135
- Cosman D, Müllberg J, Sutherland CL, Chin W, Armitage R, Fanslow W, Kubin M, Chalupny NJ (2001) ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cell cytotoxicity through the NKG2D receptor. *Immunity* 14:123–133
- Das H, Groh V, Kuijl C, Sugita M, Morita CT, Spies T, Bukowski JF (2001) MICA engagement by human V γ 2V δ 2 T cells enhances their antigen-dependent effector function. *Immunity* 15:83–93
- Diefenbach A, Jensen ER, Jamieson AM, Raulet DH (2001) Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature* 413:165–171
- Diefenbach A, Tomasello E, Lucas M, Jamieson AM, Hsia JK, Vivier E, Raulet DH (2002) Selective associations with signaling proteins determine stimulatory versus costimulatory activity of NKG2D. *Nat Immunol* 3:1142–1149
- Dobrovina ES, Dobrovina MM, Vider E, Sisson RB, O'Reilly RJ, Dupont B, Vyas YM (2003) Evasion from NK cell immunity by MHC class I-chain related molecules expressing colon carcinoma. *J Immunol* 171:6891–6899
- Dunn C, Chalupny NJ, Sutherland CL, Dosch S, Sivakumar PV, Johnson DC, Cosman D (2003) Human cytomegalovirus glycoprotein UL16 causes intracellular sequestration of NKG2D ligands, protecting against natural killer cytotoxicity. *J Exp Med* 197:1427–1439
- Eleme K, Taner SB, Onfelt B, Collinson LM, McCann FE, Chalupny NJ, Cosman D, Hopkins C, Magee AI, Davis DM (2004) Cell surface organization of stress-inducible proteins ULBP and MICA that stimulate human NK cells and T cells via NKG2D. *J Exp Med* 199:1005–1010
- Girardi M, Oppenheim DE, Steele CR, Lewis JM, Glusac E, Filler R, Hobby P, Sutton B, Tigelaar RE, Hayday AC (2001) Regulation of cutaneous malignancy by $\gamma\delta$ T cells. *Science* 294:605–609
- Groh V, Bahram S, Bauer S, Herman A, Beauchamp M, Spies T (1996) Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. *Proc Natl Acad Sci USA* 93:12445–12450
- Groh V, Steinle A, Bauer S, Spies T (1998) Recognition of stress-induced MHC molecules by intestinal epithelial $\gamma\delta$ T cells. *Science* 279:1737–1740

- Groh V, Rhinehart R, Secrist H, Bauer S, Grabstein KH, Spies T (1999) Broad tumor-associated expression and recognition by tumor-derived $\gamma\delta$ T cells of MICA and MICB. *Proc Natl Acad Sci USA* 96:6879–6884
- Groh V, Rhinehart R, Randolph-Habecker J, Topp MS, Riddell SR, Spies T (2001) Costimulation of CD8 $\alpha\beta$ T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat Immunol* 2:255–260
- Groh V, Wu J, Yee C, Spies T (2002) Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature* 419:734–738
- Groh V, Brühl A, Nelson JL, El-Gabalawi H, Spies T (2003) Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. *Proc Natl Acad Sci USA* 100, 9452–9457
- Hamerman JA, Ogasawara K, Lanier LL (2004) Toll-like receptor signaling in macrophages induces ligands for the NKG2D receptor. *J Immunol* 172:2001–2005
- Hankey KG, Drachenberg CB, Papadimitriou JC, Klassen DK, Philosophe B, Bartlett ST, Groh V, Spies T, Mann DL (2002) MIC expression in renal and pancreatic allografts. *Transplantation* 73:304–306
- Havran WL, Chien YH, Allison JP (1991) Recognition of self antigens by skin-derived T cells with invariant $\gamma\delta$ antigen receptors. *Science* 252:1430–1432
- Houchins JP, Yabe T, McSherry C, Bach FH (1991) DNA sequence analysis of NKG2, a family of related cDNA clones encoding type II integral membrane proteins on human natural killer cells. *J Exp Med* 173:1017–1020
- Hue S, Mention JJ, Monteiro RC, Zhang S, Cellier C, Schmitz J, Verkarre V, Fodil N, Bahram S, Cerf-Bensussan N, Caillat-Zucman S (2004) A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. *Immunity* 21:367–377
- Lanier LL (2001) On guard—activating NK cell receptors. *Nat Immunol* 2:23–27
- Lee JC, Lee KM, Kim DW, Heo DS (2004) Elevated TGF- β 1 secretion and down-modulation of NKG2D underlies impaired NK cytotoxicity in cancer patients. *J Immunol* 172:7335–7340
- Lee N, Llano M, Carretero M, Ishitani A, Navarro F, Lopez-Botet M, Geraghty DE (1998) HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. *Proc Natl Acad Sci USA* 95:5199–5204
- Li P, Willie ST, Bauer S, Morris DL, Spies T, Strong RK (1999) Crystal structure of the MHC class I homolog MIC-A, a $\gamma\delta$ T cell ligand. *Immunity* 10:577–584
- Li P, Morris DL, Willcox BE, Steinle A, Spies T, Strong RK (2001) Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. *Nat Immunol* 2:443–451
- Li Z, Groh V, Strong RK, Spies T (2000) A single amino acid substitution causes loss of expression of a MICA allele. *Immunogenetics* 51:246–248
- Long EO (1999) Regulation of immune responses through inhibitory receptors. *Annu Rev Immunol* 17:875–904
- McFarland BJ, Strong RK (2003) Thermodynamic analysis of degererate recognition by the NKG2D immunoreceptor: not induced fit but rigid adaptation. *Immunity* 19:803–812

- Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T, Ebert EC, Green PH, Jabri B (2004) Coordinated Induction by IL-15 of a TCR-independent pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity* 21, 357–366
- Morimoto RI, Sarge KD, Abravaya K (1992) Transcriptional regulation of heat shock genes. *J Biol Chem* 267:21987–21990
- Moser JM, Gibbs J, Jensen PE, Lukacher AE (2002) CD94-NKG2A receptors regulate antiviral CD8⁺ T cell responses. *Nat Immunol* 3:189–195
- Noppen C, Schaefer C, Zajac P, Schutz A, Kocher T, Kloth J, Heberer M, Colonna M, De Libero G, Spagnoli GC (1998) C-type lectin-like receptors in peptide-specific HLA class I-restricted expression and modulation of effector functions in clones sharing identical TCR structure and epitope specificity. *Eur J Immunol* 28:1134–1142
- Ota M, Bahram S, Katsuyama Y, Saito S, Nose Y, Sada M, Ando H, Inoko H (2000) On the MICA deleted-MICB null, HLA-B*4801 haplotype. *Tissue Antigens* 56:268–271
- Pende D, Parolini S, Pessino A, Sivori S, Augugliaro R, Morelli L, Marcenaro E, Accame L, Malaspina A, Biassoni R, Bottino C, Moretta L, Moretta A (1999) Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. *J Exp Med* 190:1505–1516
- Pessino A, Sivori S, Bottino C, Malaspina A, Morelli L, Moretta L, Biassoni R, Moretta A (1998) Molecular cloning of NKp46, a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. *J Exp Med* 188:953–960
- Ploegh HL (1998) Viral strategies of immune evasion. *Science* 280:248–253
- Radaev S, Rostro B, Brooks AG, Colonna M, Sun PD (2001) Conformational plasticity revealed by the cocrystal structure of NKG2D and its class I MHC-like ligand ULBP3. *Immunity* 15:1039–1049
- Radaev S, Kattah M, You Z, Colonna M, Sun PD (2002) Making sense of the diverse ligand recognition by NKG2D. *J Immunol* 169:6279–6285
- Raulet DH (2003) Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol* 3:781–790
- Ravetch JV, Lanier LL (2000) Immune inhibitory receptors. *Science* 290:84–89
- Roberts AI, Lee L, Schwartz E, Groh V, Spies T, Ebert EC, Jabri B (2001) NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment. *J Immunol* 167:5527–5530
- Rosen, DB, Araki, M, Hamerman JA, Chen T, Yamamura T, Lanier LL (2004) A structural basis for the association of DAP12 with mouse, but not human, NKG2D. *J Immunol* 173:2470–2478
- Russomando AK, Kikuchi M, Candia N, Franco L, Almiron M, Ubalee R, Hirayama K (2002) High frequency of MIC null haplotype (HLA-B48-MICA-del-MICB*0107N) in the Angaité Amerindian community in Paraguay. *Immunogenetics* 54:439–441
- Salih HR, Rammensee HG, Steinle A (2002) Down-regulation of MICA on human tumors by proteolytic shedding. *J Immunol* 169:4098–4102
- Salih HR, Antropius H, Gieseke F, Lutz SZ, Kanz L, Rammensee HG, Steinle A (2003) Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. *Blood* 102:1389–1396

- Siren, J, Sareneva T, Pirhonen J, Strengell M, Veckman V, Julkunen I, Matikainen S (2004) Cytokine and contact-dependent activation of natural killer cells by influenza A or Sendai virus-infected macrophages. *J. Gen Virol* 85:2357–2364
- Spada, FM, Grant EP, Peters PJ, Sugita M, Melian A, Leslie DS, Lee HK, van Donselaar E, Hanson DA, Krensky AM, Majdic O, Porcelli SA, Morita CT, Brenner MB (2000) Self-recognition of CD1 by $\gamma\delta$ T cells: implications for innate immunity. *J Exp Med* 191:937–948
- Suemizu H, Radosavljevic M, Kimura M, Sadahiro S, Yoshimura S, Bahram S, Inoko H (2002) A basolateral sorting motif in the MICA cytoplasmic tail. *Proc Natl Acad Sci USA* 99:2971–2976
- Steinle A, Groh V, Bauer S, Spies T (1998) Diversification, expression and $\gamma\delta$ T-cell recognition of evolutionary distant members of the MIC family of major histocompatibility complex class I-related molecules. *Proc Natl Acad Sci USA* 95:12510–12515
- Steinle A, Li P, Morris DL, Groh V, Lanier LL, Strong RK, Spies T (2001) Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. *Immunogenetics* 53:279–287
- Stephens HAF (2001) MICA and MICB genes: can the enigma of their polymorphism be resolved? *Trends Immunol* 22:378–385
- Sumitran-Holgersson S, Wilczek HE, Holgersson J, Soderstrom K (2002) Identification of the nonclassical HLA molecules, MICA, as targets for humoral immunity associated with irreversible rejection of kidney allografts. *Transplantation* 74:268–277
- Tieng V, Le Bouguenec C, du Merle L, Bertheau P, Desreumaux P, Janin A, Charron D, Toubert A (2002) Binding of *Escherichia coli* adhesin AfaE to CD55 triggers cell-surface expression of the MHC class I-related MICA. *Proc Natl Acad Sci USA* 5:2684–2586
- Vetter CS, Groh V, Straten P, Spies T, Bröcker E-B, Becker J. Expression of stress-induced MIC molecules on human melanoma. *J Invest Dermatol* 118, 600–605, 2002
- Welte SA, Sinsger C, Lutz SZ, Singh-Jasuja H, Sampaio KL, Eknigk U, Rammensee HG, Steinle A (2003) Selective intracellular retention of virally induced NKG2D ligands by the human cytomegalovirus UL16 glycoprotein. *Eur J Immunol* 33:194–203
- Wu J, Song Y, Bakker ABH, Bauer S, Spies T, Lanier LL, Phillips JH (1999) An activating immunoreceptor complex formed by NKG2D and DAP10. *Science* 285:730–732
- Wu J, Groh V, Spies T (2002) T cell antigen receptor engagement and specificity in the recognition of stress-inducible MIC by human epithelial $\gamma\delta$ T cells. *J Immunol* 169:1236–1240
- Wu J, Chalupny NJ, Manley TJ, Riddell SR, Cosman D, Spies T (2003) Intracellular retention of the MHC class I-related chain B ligand of NKG2D by the human CMV UL16 glycoprotein. *J Immunol* 170:4196–4200
- Yamamoto K, Fujiiyama Y, Andoh A, Bamba T, Okabe H (2001) Oxidative stress increases MICA and MICB gene expression in the human colon carcinoma cell line (CaCo-2). *Biochim Biophys Acta* 1526:10–12