Clinical & Translational Immunology

Australian and New Zealand Society for Immunology Inc.

Clinical & Translational Immunology 2020; e1230. doi: 10.1002/cti2.1230
www.wileyonlinelibrary.com/journal/cti

SPECIAL FEATURE REVIEW

NKG2D and MICA/B shedding: a 'tag game' between NK cells and malignant cells

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Received 28 October 2020;
Revised 2 December 2020;
Accepted 5 December 2020

doi: 10.1002/cti2.1230

Clinical & Translational Immunology
2020; **9**: e1230

Abstract

Natural killer (NK) cells are innate lymphocytes with cytotoxic functions and recognise target cells with the NK group 2D (NKG2D) receptor. Tumor cells are marked for NK-cell-mediated destruction upon expression of MICA and MICB (MICA/B), which are NKG2D ligands upregulated by many human cancers in response to cellular stress pathways associated with malignant transformation such as DNA damage and accumulation of misfolded proteins. However, MICA/B proteins are downregulated by tumor cells via intriguing molecular mechanisms, such as post-translational modifications in which the external domains of MICA/B are proteolytically cleaved by surface proteases and shed into the extracellular space. MICA/B shedding by cancer cells causes effective escape from NKG2D recognition and allows the development of cancers. Patients frequently have increased concentrations of soluble MICA/B molecules shed in the blood plasmas and sera, thus indicating that MICA/B shedding is a therapeutic target in immune-oncology. Here, we review the clinical significance of MICA/B shedding in cancer as well as novel immunotherapeutic approaches that aim to restore NKG2D-mediated surveillance. We also briefly discuss potential roles of MICA/B shedding beyond oncology, such as in viral infections and immune tolerance. This review will help to inform the future developments of NKG2D-based immunotherapies.

Keywords: cancer immunotherapy, MICA and MICB, NK cells, NKG2D, proteolytic shedding

INTRODUCTION

The innate immunity is the first barrier of protection against pathogens and cancers and is essential for health maintenance. Natural killer (NK) cells are innate lymphocytes specialised in recognising and killing virally infected or malignant cells. NK cells also produce cytokines, such as interferon-gamma, which promotes antigen presentation by malignant cells, and chemokines, such as XCL1 and XCL2 that recruit dendritic cells in the tumor microenvironment.[1–3] NK cell cytotoxic functions are strictly regulated by surface receptors that interact with surface ligands on target cells and trigger intracellular signalling pathways. Activating receptors promote the polarised release of cytotoxic molecules (i.e. perforin and granzymes) and cytokine production,

whereas inhibitory receptors suppress these functions.[4] NK group 2D (NKG2D) is a major activating receptor expressed by all NK cells. NKG2D is a homodimer that pairs with four copies of DNAX-activation protein 10 (DAP10), which transmit intracellular signalling via phosphatidylinositol 3-kinase and Vav1 to trigger tyrosine-phosphorylation events, calcium release, and ultimately cytotoxicity.[5–7] NKG2D-deficient mice have increased incidence of prostate carcinomas and accelerated onset of lymphomas and antibody-mediated NKG2D blockade abolishes the innate protection against cytomegalovirus infection, thus highlighting the essential role of NKG2D in the surveillance for abnormal cells.[8,9]

The expressions of NKG2D ligands are triggered by cellular stress pathways, such as DNA damage response, unfolded protein response and hypoxia.[10] As such, NKG2D ligands are frequently expressed in response to malignant transformation and are in the spotlight for cancer immunotherapy research. The human NKG2D ligands are major histocompatibility complex (MHC) class I polypeptide-related sequence A (MICA), MHC class I polypeptide-related sequence B (MICB) and the six types of UL16 binding proteins (ULBPs). MICA and MICB, here abbreviated as MICA/B, are the most studied ligands because they are downregulated by malignant cells via intriguing molecular mechanisms that are new therapeutic targets. Here, we review several of the recent and impacting works that may help to guide the development of immunotherapies for cancers. We also describe several of the alternative consequences of MICA/B downregulation, which may help to predict potential toxicities of NKG2D-based immunotherapies for cancers.

ESCAPE FROM NKG2D RECOGNITION VIA MICA/B DOWNREGULATION THROUGH PROTEOLYTIC CLEAVAGE

Solid tumors, such as melanoma and lung cancers, can be infiltrated by NK cells but at lower extents than by T cells, whereas malignant cells of haematological cancers, such as multiple myeloma and leukaemia, grow in environments (e.g. blood and bone marrow) pre-occupied by NK cells.[1,11] Intriguingly, malignant cells co-exist with NK cells in these environments without being killed. Given the essential role of NKG2D in immunosurveillance, a probable explanation is that cancer cells downregulate MICA/B to grow in an undetectable manner.[8] Here, we focus on MICA/B shedding, a major mechanism of immune escape frequently employed by human cancers.

Molecular mechanisms underlying the proteolytic cleavage of MICA/B

The MICA/B NKG2D ligands are transmembrane proteins with MHC-like extracellular domains that, in contrast to classical MHC molecules, do not associate with beta-2 microglobulin nor present antigens.[12] The ectodomains of MICA/B consist of three C-type Ig-like domains termed alpha-1, alpha-2 and alpha-3 domains. The alpha-1 and alpha-2 domains are relatively distant from the cellular membrane and serve as NKG2D binding sites, whereas the membrane-proximal alpha-3 domain does not interact with NKG2D.[13] The alpha-3 domain is where the proteolytic cleavage is initiated. First, a disulfide isomerase called ERp5 removes a disulfide bond located between the amino acid residues 202 and 259.[14] The removal of this disulfide bond likely unfolds the alpha-3 domain and exposes the proteolytic cleavage site. In fact, mutations that replace a six amino acid motif in the alpha-3 domain inhibit the shedding of MICA/B. Furthermore, the alpha-3 domain and the linear stalk in-between it and the transmembrane domain have putative proteolytic cleavage sites.[15] Upon the unfolding by ERp5, metalloproteases cut MICA/B somewhere in the stalk or close to it in the alpha-3 domain, thus releasing the entire extracellular portion of MICA/B including the alpha-1 and alpha-2 domains that are the NKG2D binding sites (Figure 1a). Of note, multiple metalloproteases have been shown to cleave MICA/B, including the membrane type matrix metalloproteinase 14 (MMP14), a disintegrin and metalloproteinase 10 (ADAM10), and ADAM17.[16,17] Therefore, MICA/B shedding is a multistep process initiated by ERp5 that enables subsequent protease-mediated cleavage.

Many cellular stress pathways that trigger MICA/B expression are known.[10] However, the cellular pathways that induce the shedding are completely unknown. Of note, extracellular matrix degradation can be mediated by MMP14 and is associated with cellular movement.[18] It is thus possible that MMP14 cleaves MICA/B during metastatic dissemination, when tumor cells

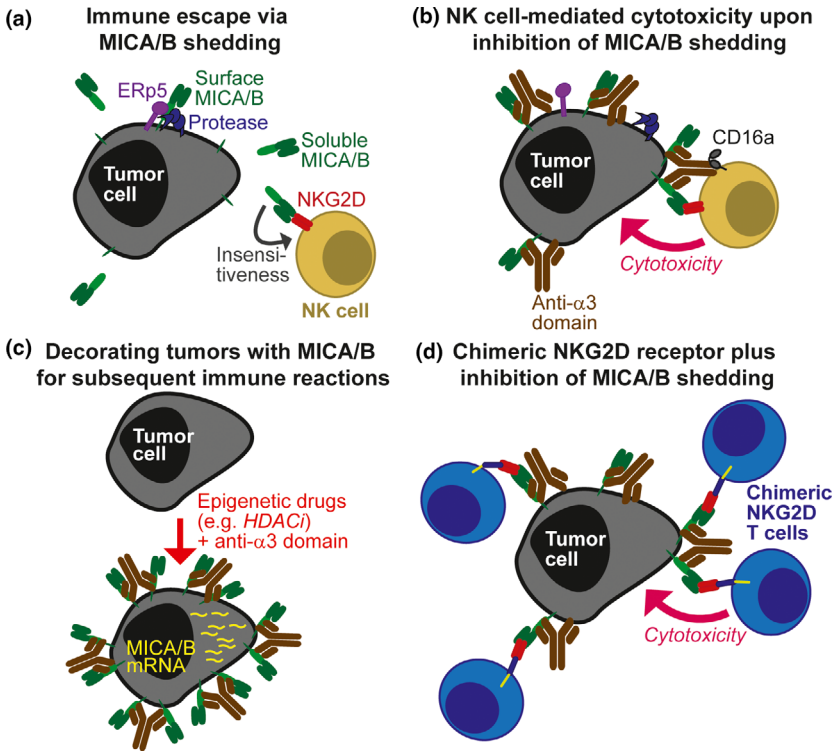

Figure 1. MICA/B shedding is an immunotherapeutic target in cancer. **(a)** Surface MICA/B proteins are cleaved in a multistep process that involves the MICA/B alpha-3 domain, ERp5 and proteases. The soluble MICA/B proteins can bind to NKG2D on NK cells and chronically inhibit them via mechanisms that are not well known yet. **(b)** MICA/B shedding can be inhibited by monoclonal antibodies that shield the alpha-3 domain and block, via steric hindrance, the interactions with ERp5 and the proteases. Consequently, these antibodies restore NKG2D recognition and trigger NK-cell-mediated killing of tumor cells. The antibodies also bind to Fc activating receptors, such as CD16a, on NK cells and trigger antibody-dependent cellular cytotoxicity. **(c)** A subset of patients have tumor cells that lack MICA/B expression, but this limitation can be bypassed with epigenetic drugs that trigger MICA/B mRNA expressions such as HDAC inhibitors (HDACi) and hypomethylating agents. The same can occur upon treatments with DNA-alkylating agents and proteasome inhibitors. These drugs can be combined with alpha-3 domain antibodies that inhibit the MICA/B shedding, thus causing high levels of surface MICA/B for subsequent immune reaction. **(d)** Chimeric NKG2D receptor T cells, here abbreviated as chimeric NKG2D T cells, are highly responsive to NKG2D ligand^{+} tumor cells, but the MICA/B shedding interferes with the efficacy of this immunotherapeutic approach. As such, inhibition of MICA/B shedding with alpha-3 domain antibodies may enable the tumor cell targeting by chimeric NKG2D T cells and promote the immunoreaction against cancers.

migrate. In addition to that, ADAM17 is commonly expressed by leukocytes and cleaves an array of receptors upon immune activation following cytokine stimulations.[19] As such, another possibility is that cancer cells of haematological malignancies also express ADAM17 and cleave MICA/B in response to cytokine stimulation. These speculations still need to be tested experimentally.

Clinical significance of MICA/B shedding in cancer

The MICA/B transmembrane proteins are converted to soluble proteins upon proteolytic cleavage. Soluble MICA/B reach the blood circulation, where they can be detected and quantified by immune assays with antibodies. These proteins are hardly detected in blood plasmas and sera of healthy individuals, but multiple studies pointed out that soluble MICA/B are increased in the blood circulation of cancer patients.[20–27] Table 1 briefly itemises the concentrations of soluble MICA/B shed in the blood circulation with specimens from a broad panel of cancer patients. Interestingly, higher levels of soluble MICA in the blood circulation of melanoma patients are associated with shorter survival following immunotherapy with T-cell checkpoint blockade, thus indicating that MICA/B shedding is a new therapeutic target in cancer immunology.[28]

Table 1. Soluble MICA/B levels are increased in the blood sera of cancer patients

Cancer	Number of samples	MICA (pg mL^{-1})	MICB (pg mL^{-1})	MICA/B (pg mL^{-1})[a]	Reference
AML	14	335	121	N.A.	21
ALL	2	435	184.5	N.A.	21
NHL	1	924	288	N.A.	21
CML	4	407	207	N.A.	21
B-cell CLL	46	~258	~109	N.A.	22
MGUS	25	100	N.D.	N.A.	24
MM	40	1980	N.D.	N.A.	24
Non-met.Prostate cancer	25	N.A.	N.A.	~2500	25
MetastaticProstate cancer	11	N.A.	N.A.	~10000	25
Melanoma	208	257.4	N.D.	N.A.	26
NSCLC	207	143.5	N.D.	N.A.	27
HD	9	34	10	N.A.	21
HD	50	90.3	N.D.	N.A.	26
HD	207	32.4	N.D.	N.A.	27

Summary of some studies that analysed the levels of MICA shed into the blood circulation of cancer patients. Healthy donors are shown for comparison.

ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; B-cell CLL, B-cell chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; *HD*, healthy donor; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; N.A., not applicable; N.D., not determined; NHL, non-Hodgkin's lymphoma; Non-met. Prostate cancer, non-metastatic prostate cancer; NSCLC, non-small-cell lung cancer.

[a]MICA was not discriminated from MICB in this indicated study.

INNOVATIVE IMMUNOTHERAPEUTIC STRATEGIES TARGETING MICA/B

Treatments that harness the immunity are called immunotherapies. They have revolutionised cancer care, and the field's pioneers (Drs James Allison and Tasuku Honjo) received the Nobel Prize in Physiology or Medicine in 2018. Most of the immunotherapeutic approaches (e.g. checkpoint blockade and adoptive cell therapy) target the adaptive immunity mediated by cytotoxic CD8 T cells. However, only a subset of patients responds to treatments whereas cancers from many responders acquire resistance.[29–31] Alternative immunotherapies with distinct mechanisms of action compared to the T-cell-based ones could effectively treat the resistant patient population. Here, we summarise some of the innovative approaches that promote the NKG2D-driven immunity for the treatment of cancers, including the ones resistant to checkpoint blockade.

Antibody-mediated inhibition of MICA/B shedding

As reviewed above, MICA/B proteins are downregulated via proteolytic cleavage that causes escape from NKG2D recognition.[14,16,17] Previous studies utilised small molecules to block ERp5 or the proteases that cleave MICA/B, with the goal of restoring NKG2D recognition of tumor cells.[14,16] However, these enzymes have broad substrate specificities, and thereby, small molecule inhibitors may block the cleavage of multiple other substrates beyond these NKG2D ligands. For example, ADAM-17 cleaves MICA/B, TNF-alpha, cytokine receptors, cell adhesion molecules and growth factors.[19] To bypass this limitation, Ferrari de Andrade *et al*. undertook an innovative approach to inhibit cleavage of MICA/B in a substrate-specific manner by constructing monoclonal antibodies that are highly specific to the MICA/B alpha-3 domain, the extracellular domain that interacts with ERp5 to initiate cleavage.[32] They first produced recombinant human alpha-3 domain and immunised mice with this peptide; then, they isolated three monoclonal antibodies that potently inhibit the MICA/B release into supernatants of tumor cell lines including melanoma, colon cancer and neuroblastoma. As a consequence of the inhibition of shedding by these antibodies, MICA/B proteins are stabilised on the surfaces of tumor cells. Of importance, the alpha-3 domain-specific antibodies do not obstruct NKG2D, which binds to the MICA/B alpha-1 and 2 domains.

One specific antibody, clone 7C6, was selected for further analyses. 7C6 substantially increases

the NK-cell-mediated killing of tumor cells in an NKG2D-dependent manner. Moreover, the Fc region of the antibody binds to CD16a receptors, an Fc activating receptor that triggers antibody-dependent cellular cytotoxicity and further contributes to NK-cell-mediated killing of tumor cells (Figure 1b). In a melanoma metastasis model with immunocompetent mice, 7C6 potently inhibits the outgrowth of melanoma in the lungs in an NK-cell-dependent manner. This model is based on lentiviral transduction of mouse melanoma cells (i.e. B16F10) to enable the expression of human MICA. Lung-infiltrating NK cells from these mice highly express cytotoxicity-related genes such as granzyme A and perforin-1, thus indicating that inhibition of MICA/B shedding induces NK cell differentiation towards a highly cytotoxic state. However, a caveat in this syngeneic melanoma model is that mice have no MICA/B genes although murine NKG2D binds to human MICA/B proteins. To bypass this limitation, Ferrari de Andrade *et al*. also established a human melanoma metastasis model with immunodeficient mice transplanted with human NK cells from healthy donors. With this model, they found that 7C6 potently inhibits metastasis formation of a human melanoma cell line in the lungs, again in an NK-cell-dependent manner.[32]

The findings described above indicate that inhibition of MICA/B shedding holds promise for cancer immunotherapy. Since 7C6 promotes NK-cell-driven immunity, this approach may serve as an alternative to cancers resistant to T-cell checkpoint blockade that harnesses mainly cytotoxic CD8 T cells. Loss of MHC class I expression or insensitivity to interferon-gamma by tumor cells can be achieved with loss-of-function mutations in the human B2M and JAK1 genes, respectively, which cause resistance to T-cell checkpoint blockers.[29–31] Ferrari de Andrade *et al*. recently demonstrated that 7C6 inhibits pre-established metastases of melanoma cell lines deficient of B2M or JAK1 and consequently prolongs the survival of immunocompetent mice.[33] As such, inhibition of MICA/B shedding may treat patients with tumors resistant to T-cell checkpoint blockers. Furthermore, NKG2D is expressed by CD8 T cells and provides co-stimulation.[34,35] Therefore, inhibition of MICA/B shedding may promote CD8 T-cell-driven immunity and enhance the therapeutic efficacy of T-cell checkpoint blockers for cancers.

Targeting soluble MICA/B to prevent NKG2D downregulation

Proteolytic cleavage removes MICA/B from cellular surfaces and generates soluble MICA/B proteins that may have biological activities. A previous study demonstrated that the soluble MICA/B proteins are immunosuppressive, because they bind to NKG2D on NK cells and CD8 T cells and cause its internalisation followed by degradation.[20] In a mouse model of prostate carcinoma with immunocompetent mice, a metastatic cell line engineered to secrete MICB forms more micrometastases in the lungs than a control cell line.[25] On the other hand, a soluble MICB-specific monoclonal antibody neutralises soluble MICB and inhibits prostate carcinoma development and metastases in mouse models.[36] This antibody also synergises with the IL-15 superagonist ALT-803, a recombinant IL-15-IL15R complex that stimulates NK cells, to inhibit prostate carcinoma in mouse models.[37] Therefore, soluble MICB produced via proteolytic cleavage is a therapeutic target.

The molecular mechanisms underlying NKG2D downregulation are partially known. Upon ligand binding, DAP10 undergoes ubiquitylation followed by endocytosis and lysosome-mediated digestion of NKG2D-DAP10 complexes. However, NKG2D downregulation via this mechanism is not necessarily a read out of immunosuppression, because NKG2D internalisation physically approximates DAP10 to ERK1/2. In fact, early endosomes containing NKG2D-DAP10 complexes following ligation co-localise with phosphorylated ERK1/2.[38] We extrapolate that soluble MICA/B differs from surface MICA/B, because the latter rapidly triggers cytotoxicity by NK cells whereas soluble MICA/B chronically downregulates NKG2D and consequently desensitises NK cells (Figure 1a). However, surface MICA/B can also desensitise NK cells upon serial engagement to target cells.[39] Therefore, NKG2D downregulation occurs upon binding to soluble and surface ligands.

Enhancing MICA/B by epigenetic therapies

The MICA and MICB genes are epigenetically regulated by methyl transferases and histone deacetylases (HDACs). Both genes are highly methylated in cell lines of human AML and peripheral blood leukocytes isolated from AML patients, and hypomethylating agents (i.e.

decitabine and azacitidine that are frequently used to treat AML) can increase MICA/B expression, which promotes NK cell degranulation against these leukaemia cells.[40] This indicates that the therapeutic efficacy of hypomethylating agents could potentially be associated with the induction of NKG2D-driven immunity. Furthermore, multiple studies showed that HDAC inhibitors potently increase MICA/B expression by cells from several cancer types, thereby promoting NK-cell-driven immunity.[41–44] In addition to epigenetic therapies, multiple studies revealed that several drugs also increase MICA/B expression by human cancers, such as poly (ADP-ribose) polymerase 1 inhibitors in AML, proteasome inhibitors in multiple myeloma, and dacarbazine in melanoma.[45–47] However, none of these drugs target the cleavage of MICA/B by proteases, and consequently, they allow MICA/B to be shed. In a previous study, Ferrari de Andrade *et al*. combined the use of panobinostat, a broad-spectrum HDAC inhibitor, with the 7C6 antibody that inhibits MICA/B shedding. They found that 7C6 stabilises the MICA/B proteins that are increased in response to HDAC inhibitor treatment. The combination of panobinostat and 7C6 reduces melanoma metastases in mice reconstituted with human NK cells.[33] This study provided a rationale for combining drugs that induce expression of MICA/B mRNA with shedding inhibitors to achieve high levels of surface MICA/B proteins (Figure 1c).

Chimeric NKG2D receptor cytotoxic cells

Adoptive transfer of cytotoxic lymphocytes is a new therapeutic modality that utilises synthetic T cells to treat cancers, and it has revolutionised the cancer immunotherapy field. T cells can be engineered to express a chimeric NKG2D receptor that is fused to the CD3ζ signalling domain, which potently induces cytotoxicity against NKG2D ligand^{+} tumor cells.[48] A phase-1 clinical trial testing this approach in patients was recently completed. In this trial, patients with leukaemia or multiple myeloma were infused with autologous chimeric NKG2D T cells. Dose-limiting toxicities, cytokine release syndrome, treatment-related neurotoxicity, autoimmunity or greater than grade 3 adverse events were all not observed, thus indicating that this NKG2D-based immunotherapy is safe. However, objective tumor responses were also not observed. For this immunotherapeutic approach to have an impact, tumor cells need to express NKG2D ligands. Soluble MICA/B proteins were detected in the blood plasmas from most of these patients, thus leading to the speculation that MICA/B shedding interfered with the NKG2D recognition of tumor cells in this trial.[49] Furthermore, soluble MICA/B might have desensitised the engineered T cells potentially by downregulating the chimeric NKG2D receptor, but this speculation has not been tested yet. Therefore, MICA/B shedding inhibitors may enhance the therapeutic efficacy of chimeric NKG2D receptor T cells (Figure 1d).

MICA/B SHEDDING BEYOND CANCER

Since MICA/B expression is triggered by stress pathways, these NKG2D ligands can be expressed by non-malignant cells under abnormal conditions. Furthermore, since proteolytic shedding of surface proteins is a common post-translational modification, the shedding can be performed by non-malignant cells that express ERp5 and proteases. Here, we extend the importance of MICA/B shedding from cancer to other human diseases, homeostasis and reproduction.

Virally infected cells express and shed MICA/B

Cells infected with various viruses display increased expression and shedding of MICA/B. During the early response to human immunodeficiency virus-1 (HIV-1) infection, T cells upregulate NKG2D ligand expression, thereby enabling NK cell recognition.[50] However, infected cells can evade NK-cell-mediated surveillance via downregulation of NKG2D ligands by the viral protein called Nef through molecular mechanisms that are not well defined yet. For example, in a previous study, 7-fold higher levels of soluble MICA were detected in the plasma of patients with chronic HIV-1 infection as compared to healthy donors.[51] Reduction of NKG2D receptor expression also occurs in NK cells from untreated patients with chronic HIV-1 infection. As mentioned earlier, high levels of soluble MICA may lead to internalisation and degradation of NKG2D receptors, resulting in the build-up of dysfunctional NK cells over time. Indeed, this aligns with the observation that many individuals with chronic HIV-1 infections accumulate, in their

bloods, dysfunctional NK cells that downregulate NKG2D and have lower cytotoxicities against target cells.[52] Therefore, HIV-infected T cells exhibit enhanced shedding of MICA, which plays a role in impairing the immunological competency of HIV-positive patients.

Increased shedding of MICA/B also occurs in cells infected with hepatitis B virus (HBV) and human cytomegalovirus (HCMV). A previous study reported that MICA expression is induced at an early stage of HBV infection and infected patients have higher levels of soluble MICA in circulation as compared to healthy controls.[53] As for HCMV, a study reports that infection with the virus triggers a significant decrease in the expression of an endogenous metalloprotease inhibitor, TIMP3, allowing for increased metalloprotease activity and enhanced shedding of MICA and other substrates. This phenomenon explains why heart transplant recipients that have developed CMV-related disease have increased levels of soluble MICA in their plasmas and reduced immunological capacities.[54] A recent study also pointed out that NK cells from patients with coronavirus disease 2019 (COVID-19) downregulate NKG2D; although this study did not investigate MICA/B shedding, soluble MICA/B proteins are frequently linked to NKG2D downregulation, and thereby, COVID-19 patients may have higher levels of soluble MICA/B shed in their blood circulations.[55] Taken together, MICA/B shedding is a conserved mechanism of immune escape that is also relevant to viral infections, and thus, the inhibition of MICA/B shedding may confer a significant benefit for patients infected with viruses such as HIV, HBV, HCMV and coronavirus.

MICA expression in the human intestine

MICA protein is also expressed by enterocytes, which are epithelial cells in the small bowel mucosa of the intestine. However, the expression is restricted to patients with coeliac disease (CD), an immunologic disorder linked to a gluten-based diet. In contrast, patients on a gluten-free diet downregulate MICA in the intestine.[56] The levels of MICA expression in the intestine increase according to disease severity, with severe enteropathy reaching the highest levels.[57] Furthermore, intraepithelial lymphocytes from CD patients express NKG2D and kill $MICA^{+}$ epithelial cell lines *in vitro*. The blood sera from patients with active CD have higher concentrations of soluble MICA compared to control and CD patients on a gluten-free diet.[56] Of note, a MICA transgenic mouse strain shows robust expressions of MICA mRNA and protein in the small intestine plus other organs including the lungs, skin and bone marrow from healthy mice.[58] The molecular mechanism that controls MICA expression in these organs is unknown, but it is expected to be associated with cellular stress. For example, enterocytes express MICA in CD potentially as a result of cellular stress caused by tissue damage and loss of tissue integrity in an environment highly colonised by microorganisms that may cause infection; this speculation still has to be verified experimentally. Therefore, MICA can be expressed by cells other than cancers, and the shedding may prevent autoimmunity against damaged organs, such as the small intestine of CD patients. As such, inhibition of MICA/B shedding, which can be achieved with alpha-3 domain-specific antibodies, may break tolerance and exacerbate this disease.[32] Caution should be taken in clinical trials testing NKG2D-based immunotherapies in cancer patients because of the potential for off-target immune reactions.

Maternal immune tolerance to the foetus via MICA/B shedding by trophoblasts

MICA/B shedding may also promote immune tolerance at the maternal–foetal interface. A previous study detected MICA/B expression in the human placenta. Specifically, immunohistochemistry revealed that MICA/B proteins are expressed by trophoblasts (a specialised cell type that forms the placenta) in the syncytiotrophoblast (an interface between the maternal uterus and placenta).[59] Freshly isolated trophoblasts express MICA/B mRNAs, and the MICA/B proteins are present on the apical and basal surfaces, as well as in intracellular vesicles that apparently contained exosomes. The sera from pregnant women are enriched with soluble MICA/B proteins, with a concentration range of 2–20 ng mL^{-1}. Soluble MICA/B in the serum was detected throughout pregnancy (from 8 to 38 gestational weeks), whereas they were not detected in sera of non-pregnant women. In addition to that, soluble MICA/B proteins were shed in supernatants from *ex vivo* cultures of placenta tissue samples, thus suggesting that soluble MICA/B in the sera of pregnant women is likely produced by the placenta. Since MICA/B was detected on the surface

and intracellular vesicles of trophoblasts, the sera of pregnant women and supernatants of *ex vivo* cultures of placenta tissues may have soluble MICA/B produced via proteolytic cleavage as well as MICA/B shed in exosomes.[59]

This previous study also reported that trophoblasts cultured *ex vivo* upregulate MICA/B in response to heat shock.[59] Of note, MICA/B shedding likely helps trophoblasts to escape NKG2D recognition by downregulating surface MICA/B. This study also reported that soluble MICA/B in the sera of pregnant women downregulates NKG2D and inhibits NK-cell-mediated cytotoxicity of K562 myeloid leukaemia cells, thus suggesting that it causes a broad immune suppression.[59] The maternal–foetus interface is highly enriched with uterine NK cells that contribute to vascular remodelling.[60] Since trophoblasts are physically close to uterine NK cells, MICA/B shedding by trophoblasts may help to prevent the maternal immune system from rejecting the placenta. As such, NKG2D-driven immunotherapies may be inappropriate for pregnant women that by unfortunate coincidence also have cancers, due to the potential of inducing immunologically mediated abortion.

CONCLUDING REMARKS

Here, we have reviewed the importance of MICA/B shedding and NKG2D-driven immunity mainly in cancers, plus brief mentions of viral infections, CD and reproduction. This review may help to inform the scientific community for the development of NKG2D-based immunotherapies for cancers, such as antibody-based therapies and adoptive T-cell therapies. We envision that these new and innovative immunotherapeutic approaches will revolutionise medicine by effectively treating cancers via mechanisms of actions that allow broad immune reactivity, since NKG2D ligands are frequently expressed by malignant cells from multiple cancer types. As such, MICA/B shedding represents a new therapeutic target in cancer immunity and thereby has the potential to cause long-lasting impacts on the cancer immunotherapy field.

However, many issues need to be considered, with the major one being the lack of evidences regarding the toxicity of NKG2D-based immunotherapies. Until now, the antibodies that inhibit MICA/B shedding or neutralise soluble MICA/B were administered to mice engrafted with MICA/B$^+$ tumors but since mice do not have the MICA/B genes they do not serve to toxicological studies.[25,32,33,36] MICA transgenic mice bypass this limitation and will be invaluable to assess the safety profile of NKG2D-based therapies.[58] Alternatively, non-human primates can also be utilised in such studies. Of note, chimeric NKG2D receptor T cells were well tolerated by patients with haematological malignancies in a clinical trial reported recently, thus serving as the first evidence of the safety of an NKG2D-based immunotherapy.[49]

It is also unknown how these therapeutic approaches perform in combination with standard treatments, such as T-cell checkpoint blockade, epigenetic therapies and radiotherapy. Furthermore, in addition to MICA/B, there are six other types of NKG2D ligands (i.e. the ULBPs) that are not cleaved but shed via exosomes.[10] Tumor cells shed ULBP-containing exosomes that downregulate NKG2D in NK cells and CD8 T cells.[61] However, it is unknown if exosome-mediated shedding lowers the surface ULBP levels. Some AML patients have leukaemia cells that simultaneously express ULBPs and MICA/B.[21] As such, ULBPs may compensate for MICA/B downregulation and enable NKG2D recognition of ULBP$^+$ tumor cells. These facts highlight the extreme complexity of NKG2D ligands, which are several and post-translationally regulated via distinct mechanisms. Nevertheless, MICA/B shedding is a new research niche in immune-oncology and offers multiple therapeutic opportunities.

ACKNOWLEDGMENTS

We thank Pedro Alves Henrique da Silva (Mount Sinai) for helpful discussions. This work was partially supported by the Jennifer J Raab Fellowship at CUNY Hunter College and the Opportunities Fund at CUNY Macaulay Honors College.

CONFLICT OF INTEREST

LFdA is co-inventor in an issued patent about an alpha-3 domain-specific antibody and serves as consultant for Cullinan Oncology. SX has no conflict of interests to declare.

AUTHOR CONTRIBUTION

Samantha Xing: Writing-original draft; Writing-review & editing. **Lucas Ferrari de Andrade:** Conceptualization; Project administration; Resources; Supervision; Writing-original draft; Writing-review & editing.

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