OncoImmunology 3, e28497; April 2014; © 2014 Landes Bioscience

Secretory pathways generating immunosuppressive NKG2D ligands

New targets for therapeutic intervention

Aroa Baragaño Raneros[1], Beatriz Suarez-Álvarez[2], and Carlos López-Larrea[1,3,*]

[1]Department of Immunology; Hospital Universitario Central de Asturias; Oviedo, Spain; [2]Cellular Biology of Renal Diseases Laboratory; Instituto de Investigación Sanitaria Fundación Jiménez Díaz; Universidad Autónoma Madrid; Madrid, Spain; [3]Fundación Renal "Iñigo Álvarez de Toledo"; Madrid, Spain

Keywords: NKs, NKG2D, soluble NKG2D ligands, shedding, exosomes

Abreviations: ADAM, a disintegrin and metalloproteinase; CLL, chronic lymphocytic leukemia; CR, complete remission; DRMs, detergent-resistant membrane microdomains; ERp5/ PDIA6, protein disulfide isomerase family A, member 6; GRP78/HSPA5, heat shock 70kDa protein 5; GTN, nitroglycerin; HIF1α, hypoxia-inducible factor 1α; IFNγ, interferon-γ; IL-1β, interleukin-1β; MICA/B, MHC class I chain-related A/B; MMP, matrix metalloprotease; MP, metalloprotease; MMPI, MMP inhibitors; NKG2D, natural killer group 2 member D; NKG2DL, NKG2D ligands; sNKG2DL, soluble NKG2DL; OS, overall survival; STAT3, signal transducer and activator of transcription 3; PDAC, pancreatic ductal adenocarcinoma; TIMP, tissue inhibitors of metalloproteinase; TFS, treatment-free survival; TGFβ, transforming growth factor-β; ULBP, UL-16 binding protein

Natural Killer Group 2 member D (NKG2D) activating receptor, present on the surface of various immune cells, plays an important role in activating the anticancer immune response by their interaction with stress-inducible NKG2D ligands (NKG2DL) on transformed cells. However, cancer cells have developed numerous mechanisms to evade the immune system via the downregulation of NKG2DL from the cell surface, including the release of NKG2DL from the cell surface in a soluble form. Here, we review the mechanisms involved in the production of soluble NKG2DL (sNKG2DL) and the potential therapeutic strategies aiming to block the release of these immunosuppressive ligands. Therapeutically enabling the NKG2D-NKG2DL interaction would promote immunorecognition of malignant cells, thus abrogating disease progression.

Introduction

NKG2D (Natural Killer Group 2 member D) is one of the most potent activating receptors expressed on the surface of natural killer (NK) cells. It is a homodimeric C-type lectin-like type II transmembrane receptor that is also expressed on other cells of the immune system, including natural killer T cells (NKT), γδ T cells, and αβ $CD8^+$ T cells.[1,2] A distinctive characteristic of NKG2D is its interaction with various human stress-inducible ligands: the transmembrane proteins MHC class I chain-related A and B (MICA and MICB), and 6 UL-16 binding proteins (ULBPs 1–6). These include GPI-anchored variants ULBP-1, -2, -3, -6, as well as transmembrane family members ULBP-4 and -5.[2,3]

The surface expression of NKG2D ligands (NKG2DL) is considered a cellular stress indicator. Whereas under physiological conditions NKG2DL expression is either absent or scarce in most cell types, the expression is enhanced during malignant transformation by the activation of different cellular pathways.[4,5] Activation of the DNA damage response pathway (ATM/ATR pathway), transcription factors (e.g., NF-κB), and various oncogenes (e.g., BCR/ABL, MYC, TP53, etc.) can enhance NKG2DL expression on the cell surface during malignant transformation, thereby exposing tumor cells to recognition and killing by NKG2D-positive immune effector cells.[6-8] Recently, Jung et al.[9] have reported that the murine ligand for NKG2D, retinoic acid early transcript 1E (*Raet1e)*, is positively regulated by E2f transcription factors (E2f1, E2f2, and E2f3). These transcriptions factors are highly expressed under oncogenic stress or growth stimulation and are involved in the regulation of the cell cycle. This finding suggests a new role for NKG2DL in cell cycle progression, and therefore, in the control of tumor development. NKG2DL are expressed in a wide range of neoplastic diseases, with preferential expression of MICA and MICB in many solid tumors whereas ULBPs are predominantly expressed in hematological malignances, as well as in gliomas and melanomas.[10] The lytic efficiency of NKG2D-positive immune effector cells is associated with the surface density of NKG2DL on the surface of the target cells.[11] Thus, high levels of NKG2DL expression result in greater immune-recognition, thereby preventing tumor progression via immune constraint.

*Correspondence to: Carlos López-Larrea; Email: inmuno@hca.es
Submitted: 01/22/2014; Revised: 02/26/2014;
Accepted: 03/10/2014; Published Online: 04/25/2014
Citation: Baragaño Raneros A, Suarez-Álvarez B, López-Larrea C. Secretory pathways generating immunosuppressive NKG2D ligands: New targets for therapeutic intervention. OncoImmunology 2014; 3:e28497; http://dx.doi.org/10.4161/onci.28497

However, malignant cells have developed a myriad of strategies to reduce or prevent NKG2DL expression.

Although NKG2DL is upregulated during malignant transformation in response to oncogenic activation, it is also known that tumor cells can evade the immune response through the inhibition of NKG2DL transcription. For example, the signal transducer and activator of transcription 3 (STAT3), a tumor-promoting transcription factor involved in several cancer-related signaling pathways (e.g., hypoxia and epithelial-mesenchymal transition) has been observed to acts as a negative regulator of *MICA* mRNA expression in HT29 colorectal cancer cells.[12] In contrast, it has been reported that the transcriptional regulatory mechanism regulating *ULBP1* mRNA levels is dictated primarily by the balance between Sp3/Sp1 transcription factors and activating enhancer binding protein 2-α (AP-2α) performing as positive and repressive regulatory factors, respectively.[13] Secretion of immunomodulatory cytokines during malignant transformation such as transforming growth factor-β (TGFβ) or interferon-γ (IFNγ) also downregulates NKG2DL transcription in tumor cell lines.[14,15]

Cells with high NKG2DL variant transcriptional levels do not always express corresponding NKG2DL proteins on the cell surface, suggesting that NKG2DL encoding genes are also subject to post-transcriptional mechanisms of regulation. Several studies have shown that NKG2DL can be downregulated by the action of multiple overexpressed cancer miRNAs that contribute to neoplastic cell avoidance of immune recognition.[16-20] Moreover, treatment with 2-deoxy-D-glucose, an inhibitor of protein N-linked glycosylation in the endoplasmic reticulum, reduces MICA/B expression on the cell surface in several tumor cell lines, suggesting that *N*-linked glycosylation is an important post-transcriptional mechanism regulating functional NKG2DL cell surface expression in cancer.[21]

In addition to these NKG2DL-attenuating mechanisms, the lack of cytotoxic NK and $CD8^+$ T-cell recognition of cancerous cells during tumor progression is also influenced by trogocytosis. During this process cell-to-cell contact allows the transference of cell membrane molecules from cancer cells to those of the immune system.[22] MICA and MICB ligands are co-transferred during this process from the tumor cell surface to the T-cell or NK-cell surface, potentially suppressing the ability of other NKG2D-positive immune cells to recognize the neoplastic cell.[23,24] However, little is definitively known about this process and further studies are needed to determine the actual impact on cancer cell immune evasion.

In addition to the myriad of immune-escape routes discussed above, the best-known mechanism of tumor escape from immunity is the release of NKG2DL from the cell surface in its soluble form. This occurrence has 2 fundamental consequences. The first is a prominent reduction of NKG2DL on the tumor cell surface, facilitating immune evasion. The second is the ability of the soluble NKG2DL (sNKG2DL) to engage the NKG2D receptor, thereby triggering its internalization. Considerable effort is being expended to understand the mechanisms involved in the production of sNKG2DL, with the aim of developing new therapeutic strategies by fostering NKG2D-NKG2DL interaction. In this review, we summarize the current knowledge regarding sNKG2DL release mechanisms and propose how the modulation of sNKG2DL by various means may stimulate immunorecognition of tumor cells, thereby preventing tumor progression.

Soluble NKG2DL In Tumor Cells

Following the discovery by Salih et al.[25] that MICA could be released in a soluble form into the extracellular milieu, several reports have since shown that NKG2DL variants are present in the serum of various cancer patients but is absent from healthy controls (**Table 1**).

NKG2DL-surface expression is highly heterogeneous among hematological cancers. While the majority of leukemia patients are positive for at least one type of NKG2DL, the combination of several distinct ligands on the cell surface is highly restricted.[26] The absence of integral NKG2DL correlates with a higher degree of release of these ligands in the soluble form, an occurrence detected mainly for MICA, MICB, and ULBP2, all of which have been found in numerous types of hematological malignancies, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), and chronic lymphocytic leukemia (CLL).[26-29]

The release of sNKG2DL has also been documented within solid tumors. In-depth analysis has revealed high levels of soluble MICA (sMICA) in cancer patient sera, including those afflicted with cervical cancer and squamous intraepithelial lesions caused by the human papilloma virus,[30] as well as hepatitis C virus-induced hepatocellular carcinoma (HCC).[31] High serum sMICA has also been detected in patients with pancreatic ductal adenocarcinoma (PDAC),[32,33] neuroblastoma,[34] gastrointestinal malignancies,[25] and melanoma.[35] Unlike MICA, little is known about the presence of other ligands in solid cancers. High levels of soluble MICB (sMICB) have been observed in PDAC patient sera[33] and in the culture media supernatant of human cervical cancer cell lines,[36] whereas elevated soluble ULBP2 (sULBP2) has been detected in melanoma[35] and non-small cell lung cancer (NSCL) patients.[37]

Release of NKG2DL from the cancer cell surface reduces their immunogenicity, thereby facilitating tumor progression. In B-cell CLL patients, despite observations that NKG2DL expression levels do not appear to correlate with disease progression, the presence of soluble forms of MICA, MICB, and ULBP2 in patient sera have been associated with poor treatment-free survival (TFS).[28] However, only sULBP2 proved to be an independent predictive factor for TFS among such leukemia patients. The presence of sMICA in Stage III and IV PDAC patient sera and the accompanying downregulation of NKG2D receptor on NK cells revealed both parameters to be independent markers of pancreatic malignant disease progression.[32] Similarly, elevated sMICB or sULBP2 levels in sera have also been associated with worse outcome, including sMICB in late-stage oral squamous cell carcinoma (OSCC)[38]

Table 1. Clinical significance of soluble NKG2DL in tumor patients.

Malignance	Soluble NKG2DL	Clinical Significance	Ref.
AML	MICA/B ULBPs 1–3	- Negative correlation with NKG2D expression. - sMICA and sULBP2 levels are associated with AML patients survival. - sULBP1 levels are lower in CR than in therapy-refractory patients.	26, 27
ALL	MICA/B ULBPs 1–3	- Negative correlation with NKG2D expression.	26, 27
CML	MICA/B, ULBPs 1–3	- Negative correlation with NKG2D expression.	26, 27
CLL	MICA/B ULBPs 1–3	- Negative correlation with NKG2D expression. - sMICA/B and sULBP2 are associated with TFS.	26–29
T-NHL	MICA/B	- No correlation with MICA/B surface expression.	26
Cervical cancer	MICA	- Negative correlation with NKG2D expression.	30
HCC	MICA	- Negative correlation with NKG2D expression. - Association with low OS and vascular invasion.	31,40
PDAC	MICA/B	- sMICA is associated with metastasis and low OS. - sMICB is associated with unresectability.	32,33
Neuroblastoma	MICA	- Negative correlation with NKG2D expression.	34
Gastrointestinal malignancies	MICA	- sMICA levels are higher in gastric, colon, and rectum cancers than healthy donors.	25
Melanoma	MICA/B, ULBP2	- sNKG2DL are associated with reduced OS. - sULBP2 is associated with disease progression and tumor load, and is an independent predictor of prognosis. - sMICB is an independent predictive factor for progression-free and OS.	35,39
NSCLC	ULBP2	- Association with low OS.	37
OSCC	MICB	- Association with low OS.	38
Multiple mieloma	MICA	- sMICA is an independent predictive factor for OS and progression-free survival.	41

Presence of soluble NKG2DL in serum from patients with different malignances is related with the evolution and prognosis of the disease. AML, Acute Myeloid Leukemia; ALL, Acute Lymphocytic Leukemia; CML, Chronic Lymphocytic Leukemia; CLL, Chronic Lymphocytic Leukemia; T-NHL, T-cell Non Hodking Lymphoma; HCC, Hepatocellular Carcinoma; PDAC, Pancreatic Ductal Adenocarcinoma; NSCLC, Non-Small-Cell Lung Cancer; OSCC, Oral Squamous Cell Carcinoma; CR, Complete Remission; TFS, Treatment-Free Survival; OS, Overall Survival.

and melanoma patients,[39] and sULBP2 among melanoma[35] and NSCL patients.[37]

Recently sNKG2DL has been shown to be not only a useful prognostic factor for malignant disease, but also a diagnostic biomarker as well. The quantification of sMICA and sMICB in the serum of PDAC patients shows an adequate sensitivity and specificity for discriminating patients from healthy donors in a similar way to carbohydrate antigen 19–9 (CA19–9), the most widely available biomarker used in the diagnosis of this disease.[33] Moreover, high levels of sMICA correlate with poor prognosis in hepatitis B virus-induced HCC patients, suggesting that assaying the sera levels of this NKG2D ligand may be useful as a predictive biomarker of the pathological course of this particular malignancy.[31]

By contrast, the status of soluble ULBP1 (sULBP1) and ULBP3 (sULBP3) molecules is obscure and further studies are needed to determine their potential role in evading the immune system and tumor progression. In short, the release of sNKG2DL during malignant transformation and its involvement in the prognosis of the disease suggest that the mechanisms involved in producing these soluble forms are potential targets that could be exploited to attenuate immune evasion and thereby reinforce antitumor immunity.

Mechanisms Involved In The Secretion Of sNKG2DL

In recent years, several distinct mechanisms have been implicated in the release of NKG2DL. Protease-mediated cleavage on the cell membrane is considered to be the main mechanism by which sMICA, sMICB, and sULBP2 are released from the cell surface whereas sULBP3 is secreted within exosomes (**Fig. 1**). However, the mechanisms related to the shedding of ULBP1 remain unknown. Alternative splicing of ULBP4 and ULBP5 produce soluble forms of these ligands, but these molecules have not been detected in primary tumors.[42,43]

MICA, MICB, and ULBP2 are cleaved by metalloproteases

There are 3 families of metalloproteases (MPs), namely matrix metalloproteases (MMPs), a disintegrin and metalloproteinases domains (ADAMs), and ADAM with thrombospondin motifs (ADAM-TS). MMPs and ADAMs have been implicated in the proteolytic shedding of NKG2DL from tumor cells. MMPs are a group of 24 human zinc-binding endopeptidases which can degrade different components of the extracellular matrix and play an important role in cancer cell survival, cell growth, angiogenesis, migration, and invasion.[44] Similar to MMPs, ADAM proteins are also salient in the pathophysiology of cancer, participating in various processes, such as the activation of positive growth

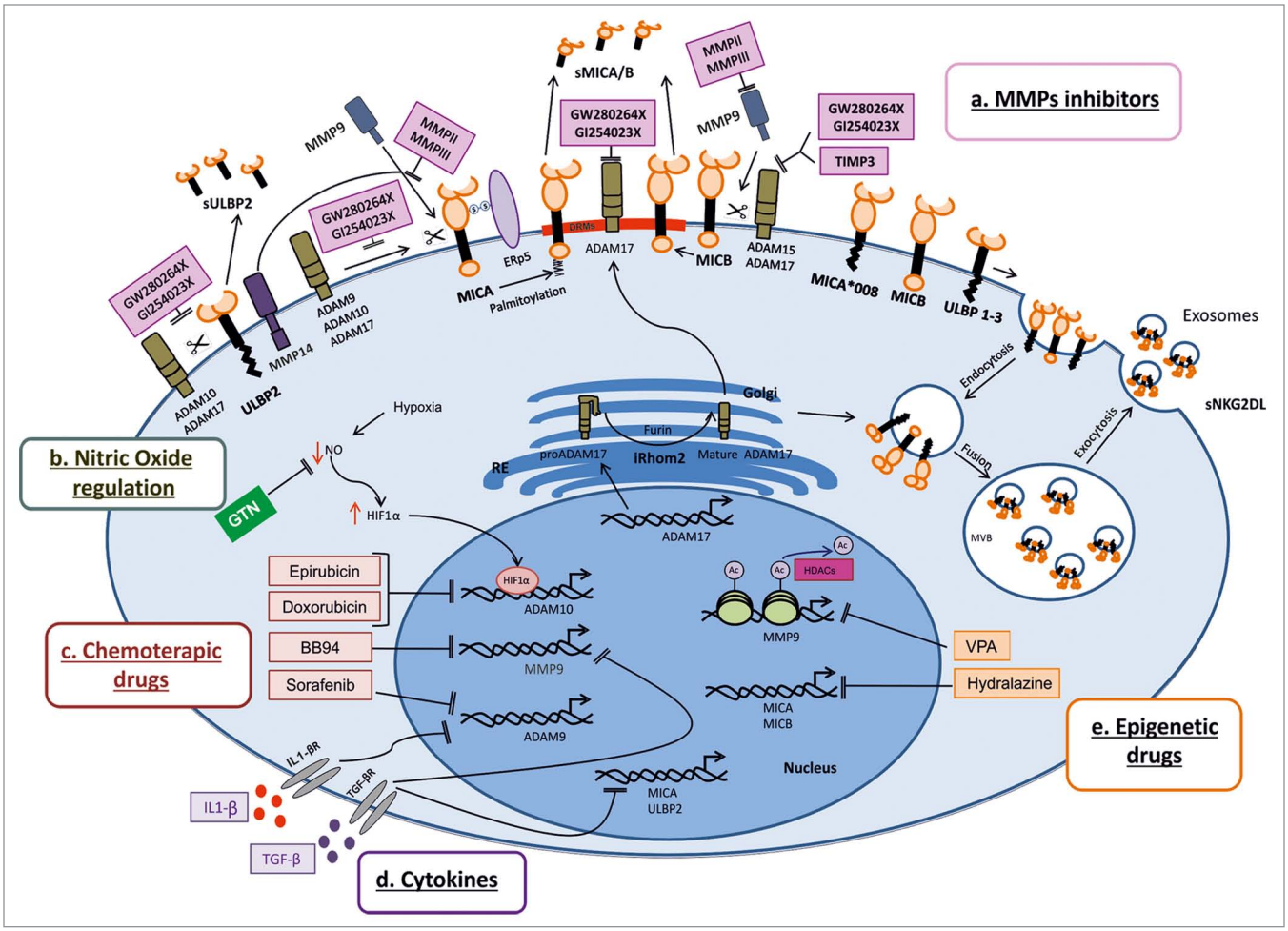

Figure 1. Mechanisms involved in the release of soluble NKG2D and blocking strategies. Natural Killer Group 2 member D ligands (NKG2DL) may be released in a soluble form (sNKG2DL) to the extracellular environment mainly through proteolytic shedding mediated by metalloproteases, or by release in exosomes derived from the cell membrane. Blockage of these mechanisms facilitates the enhanced expression of NKG2DL on the surface of tumor cells promoting immune recognition. Several therapeutic strategies have been proposed to abrogate these NK2G2DL release mechanisms. These include: (**A**) Matrix metalloproteases (MMPs) inhibitors (MMPI II, MMPI III) can inhibit shedding of MHC class I related-A/B (MICA/B), while a disintigrin and metalloproteinases domains 10 and 17 (ADAM10 and 17) inhibitors (GW280264X, GI254023X) downregulate the release of sULBP2. The natural inhibitor of ADAM17 (TIMP3) blocks ADAM17 activity, preventing MICB shedding. (**B**) During hypoxia, nitric oxide levels are reduced, promoting the upregulation of hypoxia inducible factor 1, α subunit (HIF1α). Consequently, ADAM10 mRNA levels are upregulated, correspondingly enhancing the release of sMICA and sMICB. However, the restoration of nitric oxide levels by nitroglycerin (GTN) attenuates the shedding of these ligands. (**C**) Several chemotherapeutic drugs can regulate the production of sNKG2DL through the downregulation of mRNA expression of several metalloproteases (MMP2, MMP9, ADAM10, ADAM9). (**D**) Cytokines like interleukin-1β (IL-1β) or transforming growth factor β (TGFβ) reduce shedding of NKG2DL by transcriptional regulation of ADAM9 and NKG2DL. (**E**) Epigenetic drugs such as valproate (histone deacetilase inhibitor) and hydralazine (DNA methyltransferase inhibitor) may modulate the production of sNKG2DL by downregulating MMP9 or NKG2DL mRNA expression.

factors (the EGFR/HER epidermal growth factors family), and growth inhibitory pathways (e.g., TGFβ), as well as the shedding of adhesion proteins (e.g., E-cadherin, L-selectin, ICAM-1, and VCAM) and in regulating angiogenesis.[45]

The cleavage of MICA occurs at multiple sites at the surface of tumor cells and involves several MPs that do not recognize a specific sequence motif but are more active in the proximity of the MICA α3 domain.[46] Matrix metallopeptidase 9 and 14 (MMP9 and MMP14) have been experimentally evinced to be involved in the proteolytic cleavage of MICA. Suppression of MMP9 by specific small interfering RNA (siRNA) reduces the production of sMICA from human osteosarcoma cells while the induction of these proteases by cytokines such as TGFβ promote MICA shedding from malignant glioma cell lines.[14,47-49] Similarly, inhibition of MMP14 by a short hairpin RNA (shRNA) reduces MICA shedding from murine prostate cancer cells.[50] Moreover, ADAM metallopeptidase 10 (ADAM10) and 17 (ADAM17, best known as TACE) have also been implicated in the regulation of MICA shedding. Thus, transitory RNAi-mediated silencing or pharmacological inhibition of these ADAM family MPs in MICA transfectants and prostate carcinoma cells decreases the release of soluble MICA.[46,51]

Barsoum et al.[52] showed that nitric oxide levels play an important role in the production of sMICA. During hypoxia,

nitric oxide levels are reduced, promoting the accumulation of hypoxia-inducible factor 1α (HIF1α) and enhancing ADAM10 expression, thereby increasing the release of MICA molecules. In contrast, in human prostate xenografts growing in nude mice, nitric oxide reactivation by nitroglycerin (GTN) attenuated the accumulation of HIF1α and ADAM10 expression, a molecular phenotype associated with diminishing tumor growth.[52] The inactive rhomboid protein 2 (iRHOM2), encoded by *RHBDF2* gene, has recently been reported to be a regulator of ADAM17.[53-55] This protein facilitates the maturation of ADAM17, which in turn promotes the shedding of the tumor-necrosis factor (TNF) inflammatory cytokine. However, it is not yet known whether this protein is able to regulate the production of sNKG2DL through ADAM17.

Another protease documented to be involved in MICA shedding is ADAM metallopeptidase 9 (ADAM9), a protease that generates 2 soluble MICA molecules of 39 and 37 kDa.[56] The 39 kDa protein is produced by ADAM9 proteolytic cleavage in the intracellular domain between the amino acids Gln347 and Val348, a cleavage event that generates a MICA protein isoform lacking the cytosolic domain. Subsequently, this protein undergoes a second proteolytic cleavage through the action of an unidentified protease to generate the final 37 kDa sMICA. Induction of ADAM9 expression by interleukin 1β (IL-1β) treatment in human HCC cells also promotes MICA shedding although curiously, no changes in the levels of membrane-bound MICA expression have been observed.[57] Furthermore, a positive correlation between the levels of IL-1β and sMICA was found in the serum of these patients, suggesting that this cytokine plays an important role in MICA shedding through ADAM9.[57]

Although the precise cleavage site of MICB has yet to be determined, replacement of the α3 domain with residues from HLA-A2 molecule prevents the release of sMICB and inhibits tumor development in immunodeficient mice.[58] Similar to MICA, several different MPs have been implicated in the proteolytic cleavage of the MICB ligand. MMP9 has been observed to proteolytically cleave both MICA and MICB in osteosarcoma cells (U-2 OS and SaOS-2).[59] Furthermore, it has recently been reported that ADAM metallopeptidase 15 (ADAM15) and MICB are strongly expressed in PDAC and their expression correlates with tumor stage.[60] Thus, when ADAM15 activity is blocked in PANC-1 cells, the release of sMICB is suppressed, enhancing MICB expression on the cell surface. Taken together, these observations suggest that ADAM15 plays a key role in the regulation of MICB shedding.

The proteolytic release of MICA and MICB ligands is promoted by the recruitment of these ligands to DRMs (detergent-resistant membrane microdomains). These cholesterol and sphingolipid-enriched membrane regions can recruit MICA and MICB, their proximity enhancing ADAM17 activity that subsequently promotes the efficient shedding of the ligands.[61] Knowledge of the molecular basis that associates the ligands (MICA and MICB) with cholesterol and sphingolipid-enriched domains could foster the development of new strategies for modulating the release of sNKG2DL from tumor cells. On this basis, it has been reported that MICA palmitoylation –the addition of a 16-carbon fatty acid chain by thioester linkage to cysteine residues in the cytoplasmic tail– is the prerequisite for recruiting this ligand to cholesterol-enriched microdomains. Site-directed mutagenesis in the Cys306 and Cys307 aminoacids of the MICA protein blocks palmitoylation of MICA, preventing MICA molecules from appearing in the DRM fractions. However, blockage of palmitoylation only partially reduces sMICA levels, implying that shedding does not occur exclusively from DRMs.[62]

In contrast to what happens to MICA and MICB, little is currently known in regards to the mechanisms regulating the shedding of ULBP ligands. Previous studies have demonstrated that CV1 and CHO cell lines transfected with different ligands (ULBPs1–3) release more soluble forms of ULBP2 and ULBP3 than of ULBP1.[10] These results suggest that different mechanisms may mediate the release of ULBP ligands. In support, ULBP2 has been documented to be shed from the cell membrane by the action of ADAM-family MPs (ADAM10 and ADAM17) in glioma cells and in ULBP2-transfected cells, whereas ULBP1 and ULBP3 were not released by the action of such MPs.[63,64]

TIMPs (tissue inhibitors of metalloproteinases) are endogenous inhibitors of MPs that regulate the activity of these proteases during extracellular matrix remodeling.[65] Four TIMP family members are known (TIMP1, TIMP2, TIMP3, and TIMP4), of which only TIMP1 and TIMP3 are known to act as inhibitors of MPs involved in NKG2DL shedding. TIMP1 has been observed to block the activity of MMP9, MMP14, and ADAM10, but with low affinity. By contrast, TIMP3 is a potent inhibitor of MMP9, ADAM10, and ADAM17.[66,67] Although these inhibitors can downregulate the activity of metalloproteinases involved in the shedding of MICA, MICB, and ULBP2, little is known about their direct role in the production of soluble ligands. To date, only TIMP3 has been associated with a reduction in the release of sMICB upon exogenous addition to MICB-transfected cells.[61] Therefore, an understanding of TIMP regulatory mechanisms could be useful for designing means to control the release of sNKG2DL.

ERp5 and GRP78 chaperones contribute to the release of sMICA

There is clear evidence that endoplasmic reticulum chaperones, such as thiol isomerases, are involved in the release of sMICA from the tumor cell surface. Protein disulfide isomerase family A, member 6 (PDIA6, best known as ERp5) is a member of the family of thiol isomerases that assists in the folding of nascent proteins.[29] Heat shock 70kDa protein 5 (HSPA5, best known as GRP78) is another endoplasmic reticulum protein which co-regulates protein folding mediated by the protein disulfide isomerase family, including ERp5 protein.[68] The role of ERp5 in regulating MICA shedding has been documented using different tumor cell lines treated with 5,5-dithiobis-(2-nitrobenzoic acid) and phenylarsine oxide, agents which impair protein disulfide isomerase function, or by *PDIA6* transitory silencing with siRNAs.[69] In both cases, the inhibition of ERp5 abrogates the release of sMICA, suggesting that this chaperone has a key role in regulating MICA shedding. ERp5 binds to the MICA α3 domain through transitory disulfide bonds, inducing a conformational change that is essential for the further proteolytic cleavage of MICA by MPs. The role of ERp5 in MICA shedding

has recently been reported in Hodgkin's lymphoma in which patients with high levels of ERp5 and ADAM10 expression have correspondingly high levels of sMICA in their serum.[70] Similarly, in CLL patients, high levels of sMICA are correlated with a high level of ERp5 and GRP78 expression on the cell membrane.[29]

sNKG2DL and exosomes

Exosomes are small vesicles (30–100 nm) that are released by tumor cells upon fusion of multivesicular bodies with the plasma membrane.[71] Tumors release exosomes comprising molecules that modify the tumor microenvironment, thus promoting tumor immune evasion. It has been reported that exosomes from malignant mesothelioma pleural fluid can express TGFβ and NKG2DL (MICA/B, ULBPs1–3) on their surface that can subsequently cause the downregulation of NKG2D receptor on immune cells.[72] However, others authors have shown that dendritic cell-derived exosomes express functional interleukin 15 receptor, α chain (IL-15Rα) and NKG2DL, which can promote the proliferation and activation of NK cells ex vivo.[73] Vaccination of melanoma patients with these exosomes restored the number and function of NKG2D-dependent NK cells, abrogating tumor progression in these patients. Despite these intriguing observations, it is unclear whether NKG2D modulation by exosomes may actually be due to the presence of the cytokines (i.e., TGFβ or IL15-Rα) rather than engagement through NKG2DL. Further studies are necessary to determine the exact role of exosomes bearing NKG2DL.

Although MICA, MICB, and ULBP2 are principally released by the proteolytic activity of MPs, they can also be released via exosomes, an alternative mechanism also leading immunosuppression. However, exosomes are the only known mechanism by which the GPI-anchored variants ULBP1 and ULBP3 are released from the cell surface.[10] Additionally, MICA*008, the most frequent allele in the Caucasoid population encoding a variant possessing a short transmembrane domain and a cytoplasmic domain, is preferentially released as a full-length molecule in exosomes rather than by proteolytic cleavage.[74] Ashiru O et al.[75] have recently reported that the acquisition of the GPI-domain anchor by MICA*008 is responsible for the recruitment of this allele in exosomes.

Pharmacological inhibition of NKG2DL shedding

Blockade of NKG2DL shedding by inhibition of MP activity may be a tractable strategy for enhancing antitumor immunity (Fig. 1). Release of sMICA, sMICB, and sULBP2 via MPs can be inhibited by the action of several pharmacological inhibitors of MMPs and ADAM proteins. MMP inhibitors (MMPI), including MMPI II and MMPI III are broad spectrum inhibitors of several MPs and application of these MMPIs have been shown to downregulate MICA and ULBP2 shedding in C1R-MICA and C1R-ULBP2 transfectants, respectively.[46,63] Furthermore, treatments with ADAM10 and ADAM17 inhibitors (GW280264X and GI254023X) has also been observed to reduce the release of sMICA in C1R-MICA transfectants.[46] Nevertheless, these synthetic inhibitors have the disadvantage of being relatively nonspecific, prompting the evaluation of new approaches using specific natural TIMPs inhibitors.

Chemotherapy treatments can modulate the production of sNKG2DL. Some genotoxic treatments such as gemcitabine, a first-line treatment used in pancreatic cancer, may increase the release of soluble ligands due to the upregulation of NKG2DL expression on the cell surface, a phenomenon documented in studies of pancreatic cancer cells.[76,77] However, other chemotherapeutics may reduce the level of sNKG2DL by blocking MP activity. BB94 (Batismatat), an agent that inhibits both MMPs and ADAMs, has been observed to dampen the production of sNKG2DL (MICA, sMICB, and sULBP2).[46,61,63] Epirubicin and doxorubicin are anthracycline antitumor drugs commonly used in HCC chemotherapy. Treatment with these drugs in HCC cell lines (HepG2 and PLC/PRF/5 cell lines) inhibits ADAM10 expression, increasing the expression of membrane-bound MICA and correspondingly decreasing the release of sMICA.[78] Sorafenib, an inhibitor of angiogenesis that effectively prolongs the median overall survival of patients with advanced HCC, yields similar results through the downregulation of ADAM9.[56] Thus, various chemotherapy reagents can inhibit the proteolytic activity of several MPs, increasing the surface expression of NKG2DL and enhancing malignant cell recognition (and potentially elimination) by the immune system.

Epigenetic modifying drugs, such as histone deacetylase inhibitors and demethylating reagents, have also been observed to increase NKG2DL expression on the cell surface of various cancer cell types. Valproate treatment in osteosarcoma cells downregulates MMP9 expression and thereby enhances the expression of MICA and MICB ligands on the cell surface while downregulating the release of soluble forms of these ligands.[79] Moreover, the hypomethylating reagent hydralazine reduces the release of soluble form of MICA and MICB in conjunction with enhanced surface expression of these ligands.[80] These observations suggest that epigenetic drugs could be a new therapeutic strategy to enhance the immunorecognition of tumor cells, not only by promoting NKG2DL expression on the cancer cell surface, but also by reducing the release of the soluble forms of these ligands.

Concluding Remarks

In recent years, the release of soluble forms of NKG2DLs has been extensively investigated with regard to their involvement in tumor pathologies. Several studies have shown that sNKG2DL are frequently associated with a worse prognosis among tumor patients. This corollary may be due to the interaction of soluble ligands with the NKG2D receptor, a contact that promotes receptor internalization and corresponding reduced cytotoxic NK or $CD8^+$ T-cell recognition of cancerous cells. Therefore, modulation of the balance between soluble and membrane-bound NKG2DL could be useful in the development of anticancer therapy. In these regards, it has been reported that specific inhibitors of MP activity can block the production of sNKG2DL, although future studies will be required to determine whether these protease inhibitors comprise a valid therapeutic strategy for modulating the release of sNKG2DL. Finally, the recently elucidated role of exosomes in cancer development and in immunosuppression via NKG2DL shedding suggests that further knowledge delineating the mechanisms by which

exosomes are released will further endeavors to develop new strategies aiming to enhance immunity through the NKG2D-NKG2DL interaction.

In conclusion, although it is widely accepted that the presence of sNKG2DL is closely related to the prognosis of tumor, in-depth knowledge of the mechanisms involved in the release of these soluble forms will allow us to address new therapeutic approaches for enhancing the immune recognition of tumor cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by Spanish grant PI12/02587 and the Red de Investigación Renal (REDiNREN RD12/0021/0021) from the Instituto de Salud Carlos III, and by European Union "Fondos FEDER".

References

1. González S, Groh V, Spies T. Immunobiology of human NKG2D and its ligands. Curr Top Microbiol Immunol 2006; 298:121-38; PMID:16329186; http://dx.doi.org/10.1007/3-540-27743-9_6
2. López-Larrea C, Suárez-Alvarez B, López-Soto A, López-Vázquez A, Gonzalez S. The NKG2D receptor: sensing stressed cells. Trends Mol Med 2008; 14:179-89; http://dx.doi.org/10.1016/j.molmed.2008.02.004; PMID:18353724
3. Eagle RA, Traherne JA, Hair JR, Jafferji I, Trowsdale J. ULBP6/RAET1L is an additional human NKG2D ligand. Eur J Immunol 2009; 39:3207-16; http://dx.doi.org/10.1002/eji.200939502; PMID:19658097
4. Ullrich E, Koch J, Cerwenka A, Steinle A. New prospects on the NKG2D/NKG2DL system for oncology. Oncoimmunology 2013; 2:e26097; PMID:24353908; http://dx.doi.org/10.4161/onci.26097
5. Raulet DH, Gasser S, Gowen BG, Deng W, Jung H. Regulation of ligands for the NKG2D activating receptor. Annu Rev Immunol 2013; 31:413-41; http://dx.doi.org/10.1146/annurev-immunol-032712-095951; PMID:23298206
6. Heinemann A, Zhao F, Pechlivanis S, Eberle J, Steinle A, Diederichs S, Schadendorf D, Paschen A. Tumor suppressive microRNAs miR-34a/c control cancer cell expression of ULBP2, a stress-induced ligand of the natural killer cell receptor NKG2D. Cancer Res 2012; 72:460-71; http://dx.doi.org/10.1158/0008-5472.CAN-11-1977; PMID:22102694
7. Terme M, Borg C, Guilhot F, Masurier C, Flament C, Wagner EF, Caillat-Zucman S, Bernheim A, Turhan AG, Caignard A, et al. BCR/ABL promotes dendritic cell-mediated natural killer cell activation. Cancer Res 2005; 65:6409-17; PMID:16024645; http://dx.doi.org/10.1158/0008-5472.CAN-04-2675
8. Unni AM, Bondar T, Medzhitov R. Intrinsic sensor of oncogenic transformation induces a signal for innate immunosurveillance. Proc Natl Acad Sci U S A 2008; 105:1686-91; http://dx.doi.org/10.1073/pnas.0701675105; PMID:18223157
9. Jung H, Hsiung B, Pestal K, Procyk E, Raulet DH. RAE-1 ligands for the NKG2D receptor are regulated by E2F transcription factors, which control cell cycle entry. J Exp Med 2012; 209:2409-22; http://dx.doi.org/10.1084/jem.20120565; PMID:23166357
10. Fernández-Messina L, Ashiru O, Boutet P, Agüera-González S, Skepper JN, Reyburn HT, Valés-Gómez M. Differential mechanisms of shedding of the glycosylphosphatidylinositol (GPI)-anchored NKG2D ligands. J Biol Chem 2010; 285:8543-51; http://dx.doi.org/10.1074/jbc.M109.045906; PMID:20080967
11. Pende D, Rivera P, Marcenaro S, Chang CC, Biassoni R, Conte R, Kubin M, Cosman D, Ferrone S, Moretta L, et al. Major histocompatibility complex class I-related chain A and UL16-binding protein expression on tumor cell lines of different histotypes: analysis of tumor susceptibility to NKG2D-dependent natural killer cell cytotoxicity. Cancer Res 2002; 62:6178-86; PMID:12414645
12. Bedel R, Thiery-Vuillemin A, Grandclement C, Balland J, Remy-Martin JP, Kantelip B, Pallandre JR, Pivot X, Ferrand C, Tiberghien P, et al. Novel role for STAT3 in transcriptional regulation of NK immune cell targeting receptor MICA on cancer cells. Cancer Res 2011; 71:1615-26; http://dx.doi.org/10.1158/0008-5472.CAN-09-4540; PMID:21257710
13. López-Soto A, Quiñones-Lombraña A, López-Arbesú R, López-Larrea C, González S. Transcriptional regulation of ULBP1, a human ligand of the NKG2D receptor. J Biol Chem 2006; 281:30419-30; PMID:16901903; http://dx.doi.org/10.1074/jbc.M604868200
14. Eisele G, Wischhusen J, Mittelbronn M, Meyermann R, Waldhauer I, Steinle A, Weller M, Friese MA. TGF-beta and metalloproteinases differentially suppress NKG2D ligand surface expression on malignant glioma cells. Brain 2006; 129:2416-25; PMID:16891318; http://dx.doi.org/10.1093/brain/awl205
15. Schwinn N, Vokhminova D, Sucker A, Textor S, Striegel S, Moll I, Nausch N, Tuettenberg J, Steinle A, Cerwenka A, et al. Interferon-gamma down-regulates NKG2D ligand expression and impairs the NKG2D-mediated cytolysis of MHC class I-deficient melanoma by natural killer cells. Int J Cancer 2009; 124:1594-604; http://dx.doi.org/10.1002/ijc.24098; PMID:19089914
16. Himmelreich H, Mathys A, Wodnar-Filipowicz A, Kalberer CP. Post-transcriptional regulation of ULBP1 ligand for the activating immunoreceptor NKG2D involves 3′ untranslated region. Hum Immunol 2011; 72:470-8; http://dx.doi.org/10.1016/j.humimm.2011.03.005; PMID:21406206
17. Nachmani D, Lankry D, Wolf DG, Mandelboim O. The human cytomegalovirus microRNA miR-UL112 acts synergistically with a cellular microRNA to escape immune elimination. Nat Immunol 2010; 11:806-13; http://dx.doi.org/10.1038/ni.1916; PMID:20694010
18. Stern-Ginossar N, Gur C, Biton M, Horwitz E, Elboim M, Stanietsky N, Mandelboim M, Mandelboim O. Human microRNAs regulate stress-induced immune responses mediated by the receptor NKG2D. Nat Immunol 2008; 9:1065-73; http://dx.doi.org/10.1038/ni.1642; PMID:18677316
19. Yadav D, Ngolab J, Lim RS, Krishnamurthy S, Bui JD. Cutting edge: down-regulation of MHC class I-related chain A on tumor cells by IFN-gamma-induced microRNA. J Immunol 2009; 182:39-43; PMID:19109132; http://dx.doi.org/10.4049/jimmunol.182.1.39
20. Tsukerman P, Stern-Ginossar N, Gur C, Glasner A, Nachmani D, Bauman Y, Yamin R, Vitenshtein A, Stanietsky N, Bar-Mag T, et al. MiR-10b downregulates the stress-induced cell surface molecule MICB, a critical ligand for cancer cell recognition by natural killer cells. Cancer Res 2012; 72:5463-72; http://dx.doi.org/10.1158/0008-5472.CAN-11-2671; PMID:22915757
21. Andresen L, Skovbakke SL, Persson G, Hagemann-Jensen M, Hansen KA, Jensen H, Skov S. 2-deoxy D-glucose prevents cell surface expression of NKG2D ligands through inhibition of N-linked glycosylation. J Immunol 2012; 188:1847-55; http://dx.doi.org/10.4049/jimmunol.1004085; PMID:22227571
22. Caumartin J, Favier B, Daouya M, Guillard C, Moreau P, Carosella ED, LeMaoult J. Trogocytosis-based generation of suppressive NK cells. EMBO J 2007; 26:1423-33; PMID:17318190; http://dx.doi.org/10.1038/sj.emboj.7601570
23. Roda-Navarro P, Vales-Gomez M, Chisholm SE, Reyburn HT. Transfer of NKG2D and MICB at the cytotoxic NK cell immune synapse correlates with a reduction in NK cell cytotoxic function. Proc Natl Acad Sci U S A 2006; 103:11258-63; PMID:16849432; http://dx.doi.org/10.1073/pnas.0600721103
24. Domaica CI, Fuertes MB, Rossi LE, Girart MV, Avila DE, Rabinovich GA, Zwirner NW. Tumour-experienced T cells promote NK cell activity through trogocytosis of NKG2D and NKp46 ligands. EMBO Rep 2009; 10:908-15; http://dx.doi.org/10.1038/embor.2009.92; PMID:19498463
25. Salih HR, Rammensee HG, Steinle A. Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. J Immunol 2002; 169:4098-102; PMID:12370336; http://dx.doi.org/10.4049/jimmunol.169.8.4098
26. Salih HR, Antropius H, Gieseke F, Lutz SZ, Kanz L, Rammensee HG, Steinle A. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood 2003; 102:1389-96; PMID:12714493; http://dx.doi.org/10.1182/blood-2003-01-0019
27. Hilpert J, Grosse-Hovest L, Grünebach F, Buechele C, Nuebling T, Raum T, Steinle A, Salih HR. Comprehensive analysis of NKG2D ligand expression and release in leukemia: implications for NKG2D-mediated NK cell responses. J Immunol 2012; 189:1360-71; http://dx.doi.org/10.4049/jimmunol.1200796; PMID:22730533
28. Nückel H, Switala M, Sellmann L, Horn PA, Dürig J, Dührsen U, Küppers R, Grosse-Wilde H, Rebmann V. The prognostic significance of soluble NKG2D ligands in B-cell chronic lymphocytic leukemia. Leukemia 2010; 24:1152-9; http://dx.doi.org/10.1038/leu.2010.74; PMID:20428196
29. Huergo-Zapico L, Gonzalez-Rodriguez AP, Contesti J, Gonzalez E, López-Soto A, Fernandez-Guizan A, Acebes-Huerta A, de Los Toyos JR, Lopez-Larrea C, Groh V, et al. Expression of ERp5 and GRP78 on the membrane of chronic lymphocytic leukemia cells: association with soluble MICA shedding. Cancer Immunol Immunother 2012; 61:1201-10; http://dx.doi.org/10.1007/s00262-011-1195-z; PMID:22215138
30. Arreygue-Garcia NA, Daneri-Navarro A, del Toro-Arreola A, Cid-Arregui A, Gonzalez-Ramella O, Jave-Suarez LF, Aguilar-Lemarroy A, Troyo-Sanroman R, Bravo-Cuellar A, Delgado-Rizo V, et al. Augmented serum level of major histocompatibility complex class I-related chain A (MICA) protein and reduced NKG2D expression on NK and T cells in patients with cervical cancer and precursor lesions. BMC Cancer 2008; 8:16; http://dx.doi.org/10.1186/1471-2407-8-16; PMID:18208618

31. Kumar V, Yi Lo PH, Sawai H, Kato N, Takahashi A, Deng Z, Urabe Y, Mbarek H, Tokunaga K, Tanaka Y, et al. Soluble MICA and a MICA variation as possible prognostic biomarkers for HBV-induced hepatocellular carcinoma. PLoS One 2012; 7:e44743; PMID:23024757; http://dx.doi.org/10.1371/journal.pone.0044743
32. Duan X, Deng L, Chen X, Lu Y, Zhang Q, Zhang K, Hu Y, Zeng J, Sun W. Clinical significance of the immunostimulatory MHC class I chain-related molecule A and NKG2D receptor on NK cells in pancreatic cancer. Med Oncol 2011; 28:466-74; http://dx.doi.org/10.1007/s12032-010-9480-9; PMID:20354827
33. Chung HW, Lim JB. Clinical significance of serum levels of immune-associated molecules, uric acid and soluble MHC class I chain-related molecules A and B, as diagnostic tumor markers for pancreatic ductal adenocarcinoma. Cancer Sci 2011; 102:1673-9; http://dx.doi.org/10.1111/j.1349-7006.2011.01989.x; PMID:21615621
34. Raffaghello L, Prigione I, Airoldi I, Camoriano M, Levreri I, Gambini C, Pende D, Steinle A, Ferrone S, Pistoia V. Downregulation and/or release of NKG2D ligands as immune evasion strategy of human neuroblastoma. Neoplasia 2004; 6:558-68; PMID:15548365; http://dx.doi.org/10.1593/neo.04316
35. Paschen A, Sucker A, Hill B, Moll I, Zapatka M, Nguyen XD, Sim GC, Gutmann I, Hassel J, Becker JC, et al. Differential clinical significance of individual NKG2D ligands in melanoma: soluble ULBP2 as an indicator of poor prognosis superior to S100B. Clin Cancer Res 2009; 15:5208-15; http://dx.doi.org/10.1158/1078-0432.CCR-09-0886; PMID:19671853
36. Del Toro-Arreola S, Arreygue-Garcia N, Aguilar-Lemarroy A, Cid-Arregui A, Jimenez-Perez M, Haramati J, Barros-Nuñez P, Gonzalez-Ramella O, Del Toro-Arreola A, Ortiz-Lazareno P, et al. MHC class I-related chain A and B ligands are differentially expressed in human cervical cancer cell lines. Cancer Cell Int 2011; 11:15; http://dx.doi.org/10.1186/1475-2867-11-15; PMID:21631944
37. Yamaguchi K, Chikumi H, Shimizu A, Takata M, Kinoshita N, Hashimoto K, Nakamoto M, Matsunaga S, Kurai J, Miyake N, et al. Diagnostic and prognostic impact of serum-soluble UL16-binding protein 2 in lung cancer patients. Cancer Sci 2012; 103:1405-13; http://dx.doi.org/10.1111/j.1349-7006.2012.02330.x; PMID:22587355
38. Tamaki S, Kawakami M, Ishitani A, Kawashima W, Kasuda S, Yamanaka Y, Shimomura H, Imai Y, Nakagawa Y, Hatake K, et al. Soluble MICB serum levels correlate with disease stage and survival rate in patients with oral squamous cell carcinoma. Anticancer Res 2010; 30:4097-101; PMID:21036725
39. Wu BJ, Li WP, Qian C, Ding W, Zhou ZW, Jiang H. Serum soluble MICB (sMICB) correlates with disease progression and survival in melanoma patients. Tumour Biol 2013; 34:565-9; http://dx.doi.org/10.1007/s13277-012-0582-1; PMID:23150178
40. Jinushi M, Takehara T, Tatsumi T, Hiramatsu N, Sakamori R, Yamaguchi S, Hayashi N. Impairment of natural killer cell and dendritic cell functions by the soluble form of MHC class I-related chain A in advanced human hepatocellular carcinomas. J Hepatol 2005; 43:1013-20; PMID:16168521; http://dx.doi.org/10.1016/j.jhep.2005.05.026
41. Rebmann V, Schütt P, Brandhorst D, Opalka B, Moritz T, Nowrousian MR, Grosse-Wilde H. Soluble MICA as an independent prognostic factor for the overall survival and progression-free survival of multiple myeloma patients. Clin Immunol 2007; 123:114-20; PMID:17218152; http://dx.doi.org/10.1016/j.clim.2006.11.007
42. Cao W, Xi X, Hao Z, Li W, Kong Y, Cui L, Ma C, Ba D, He W. RAET1E2, a soluble isoform of the UL16-binding protein RAET1E produced by tumor cells, inhibits NKG2D-mediated NK cytotoxicity. J Biol Chem 2007; 282:18922-8; PMID:17470428; http://dx.doi.org/10.1074/jbc.M702504200
43. Eagle RA, Flack G, Warford A, Martínez-Borra J, Jafferji I, Traherne JA, Ohashi M, Boyle LH, Barrow AD, Caillat-Zucman S, et al. Cellular expression, trafficking, and function of two isoforms of human ULBP5/RAET1G. PLoS One 2009; 4:e4503; http://dx.doi.org/10.1371/journal.pone.0004503; PMID:19223974
44. Noël A, Gutiérrez-Fernández A, Sounni NE, Behrendt N, Maquoi E, Lund IK, Cal S, Hoyer-Hansen G, López-Otín C. New and paradoxical roles of matrix metalloproteinases in the tumor microenvironment. Front Pharmacol 2012; 3:140; http://dx.doi.org/10.3389/fphar.2012.00140; PMID:22822400
45. Duffy MJ, Mullooly M, O'Donovan N, Sukor S, Crown J, Pierce A, McGowan PM. The ADAMs family of proteases: new biomarkers and therapeutic targets for cancer? Clin Proteomics 2011; 8:9; http://dx.doi.org/10.1186/1559-0275-8-9; PMID:21906355
46. Waldhauer I, Goehlsdorf D, Gieseke F, Weinschenk T, Wittenbrink M, Ludwig A, Stevanovic S, Rammensee HG, Steinle A. Tumor-associated MICA is shed by ADAM proteases. Cancer Res 2008; 68:6368-76; http://dx.doi.org/10.1158/0008-5472.CAN-07-6768; PMID:18676862
47. Sun D, Wang X, Zhang H, Deng L, Zhang Y. MMP9 mediates MICA shedding in human osteosarcomas. Cell Biol Int 2011; 35:569-74; http://dx.doi.org/10.1042/CBI20100431; PMID:21143201
48. Yamanegi K, Yamane J, Kobayashi K, Ohyama H, Nakasho K, Yamada N, Hata M, Fukunaga S, Futani H, Okamura H, et al. Downregulation of matrix metalloproteinase-9 mRNA by valproic acid plays a role in inhibiting the shedding of MHC class I-related molecules A and B on the surface of human osteosarcoma cells. Oncol Rep 2012; 28:1585-90; http://dx.doi.org/10.3892/or.2012.1981; PMID:22923031
49. Kim ES, Kim MS, Moon A. TGF-beta-induced upregulation of MMP-2 and MMP-9 depends on p38 MAPK, but not ERK signaling in MCF10A human breast epithelial cells. Int J Oncol 2004; 25:1375-82; PMID:15492828
50. Liu G, Atteridge CL, Wang X, Lundgren AD, Wu JD. The membrane type matrix metalloproteinase MMP14 mediates constitutive shedding of MHC class I chain-related molecule A independent of A disintegrin and metalloproteinases. J Immunol 2010; 184:3346-50; http://dx.doi.org/10.4049/jimmunol.0903789; PMID:20208009
51. Chitadze G, Lettau M, Bhat J, Wesch D, Steinle A, Fürst D, Mytilineos J, Kalthoff H, Janssen O, Oberg HH, et al. Shedding of endogenous MHC class I-related chain molecules A and B from different human tumor entities: heterogeneous involvement of the "a disintegrin and metalloproteases" 10 and 17. Int J Cancer 2013; 133:1557-66; http://dx.doi.org/10.1002/ijc.28174; PMID:23526433
52. Barsoum IB, Hamilton TK, Li X, Cotechini T, Miles EA, Siemens DR, Graham CH. Hypoxia induces escape from innate immunity in cancer cells via increased expression of ADAM10: role of nitric oxide. Cancer Res 2011; 71:7433-41; http://dx.doi.org/10.1158/0008-5472.CAN-11-2104; PMID:22006996
53. Adrain C, Zettl M, Christova Y, Taylor N, Freeman M. Tumor necrosis factor signaling requires iRhom2 to promote trafficking and activation of TACE. Science 2012; 335:225-8; http://dx.doi.org/10.1126/science.1214400; PMID:22246777
54. McIlwain DR, Lang PA, Maretzky T, Hamada K, Ohishi K, Maney SK, Berger T, Murthy A, Duncan G, Xu HC, et al. iRhom2 regulation of TACE controls TNF-mediated protection against Listeria and responses to LPS. Science 2012; 335:229-32; http://dx.doi.org/10.1126/science.1214448; PMID:22246778
55. Siggs OM, Xiao N, Wang Y, Shi H, Tomisato W, Li X, Xia Y, Beutler B. iRhom2 is required for the secretion of mouse TNFα. Blood 2012; 119:5769-71; http://dx.doi.org/10.1182/blood-2012-03-417949; PMID:22550345
56. Kohga K, Takehara T, Tatsumi T, Ishida H, Miyagi T, Hosui A, Hayashi N. Sorafenib inhibits the shedding of major histocompatibility complex class I-related chain A on hepatocellular carcinoma cells by down-regulating a disintegrin and metalloproteinase 9. Hepatology 2010; 51:1264-73; http://dx.doi.org/10.1002/hep.23456; PMID:20099300
57. Kohga K, Tatsumi T, Tsunematsu H, Aono S, Shimizu S, Kodama T, Hikita H, Yamamoto M, Oze T, Aketa H, et al. Interleukin-1β enhances the production of soluble MICA in human hepatocellular carcinoma. Cancer Immunol Immunother 2012; 61:1425-32; PMID:22302133; http://dx.doi.org/10.1007/s00262-012-1208-6
58. Wu JD, Atteridge CL, Wang X, Seya T, Plymate SR. Obstructing shedding of the immunostimulatory MHC class I chain-related gene B prevents tumor formation. Clin Cancer Res 2009; 15:632-40; http://dx.doi.org/10.1158/1078-0432.CCR-08-1305; PMID:19147769
59. Yamanegi K, Yamane J, Kobayashi K, Kato-Kogoe N, Ohyama H, Nakasho K, Yamada N, Hata M, Fukunaga S, Futani H, et al. Valproic acid cooperates with hydralazine to augment the susceptibility of human osteosarcoma cells to Fas- and NK cell-mediated cell death. Int J Oncol 2012; 41:83-91; http://dx.doi.org/10.3892/ijo.2012.1438; PMID:22576685
60. Duan X, Mao X, Sun W. ADAM15 is involved in MICB shedding and mediates the effects of gemcitabine on MICB shedding in PANC-1 pancreatic cancer cells. Mol Med Rep 2013; 7:991-7; http://dx.doi.org/10.3892/mmr.2013.1272; PMID:23314034
61. Boutet P, Agüera-González S, Atkinson S, Pennington CJ, Edwards DR, Murphy G, Reyburn HT, Valés-Gómez M. Cutting edge: the metalloproteinase ADAM17/TNF-alpha-converting enzyme regulates proteolytic shedding of the MHC class I-related chain B protein. J Immunol 2009; 182:49-53; PMID:19109134; http://dx.doi.org/10.4049/jimmunol.182.1.49
62. Agüera-González S, Gross CC, Fernández-Messina L, Ashiru O, Esteso G, Hang HC, Reyburn HT, Long EO, Valés-Gómez M. Palmitoylation of MICA, a ligand for NKG2D, mediates its recruitment to membrane microdomains and promotes its shedding. Eur J Immunol 2011; 41:3667-76; PMID:21928280; http://dx.doi.org/10.1002/eji.201141645
63. Waldhauer I, Steinle A. Proteolytic release of soluble UL16-binding protein 2 from tumor cells. Cancer Res 2006; 66:2520-6; PMID:16510567; http://dx.doi.org/10.1158/0008-5472.CAN-05-2520
64. Hedlund M, Nagaeva O, Kargl D, Baranov V, Mincheva-Nilsson L. Thermal- and oxidative stress causes enhanced release of NKG2D ligand-bearing immunosuppressive exosomes in leukemia/lymphoma T and B cells. PLoS One 2011; 6:e16899; http://dx.doi.org/10.1371/journal.pone.0016899; PMID:21364924
65. Baker AH, Edwards DR, Murphy G. Metalloproteinase inhibitors: biological actions and therapeutic opportunities. J Cell Sci 2002; 115:3719-27; PMID:12235282; http://dx.doi.org/10.1242/jcs.00063

66. Bourboulia D, Stetler-Stevenson WG. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs): Positive and negative regulators in tumor cell adhesion. Semin Cancer Biol 2010; 20:161-8; http://dx.doi.org/10.1016/j.semcancer.2010.05.002; PMID:20470890
67. Brew K, Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. Biochim Biophys Acta 2010; 1803:55-71; http://dx.doi.org/10.1016/j.bbamcr.2010.01.003; PMID:20080133
68. Zhang LH, Zhang X. Roles of GRP78 in physiology and cancer. J Cell Biochem 2010; 110:1299-305; http://dx.doi.org/10.1002/jcb.22679; PMID:20506407
69. Kaiser BK, Yim D, Chow IT, Gonzalez S, Dai Z, Mann HH, Strong RK, Groh V, Spies T. Disulphide-isomerase-enabled shedding of tumour-associated NKG2D ligands. Nature 2007; 447:482-6; PMID:17495932; http://dx.doi.org/10.1038/nature05768
70. Zocchi MR, Catellani S, Canevali P, Tavella S, Garuti A, Villaggio B, Zunino A, Gobbi M, Fraternali-Orcioni G, Kunkl A, et al. High ERp5/ADAM10 expression in lymph node microenvironment and impaired NKG2D ligands recognition in Hodgkin lymphomas. Blood 2012; 119:1479-89; http://dx.doi.org/10.1182/blood-2011-07-370841; PMID:22167753
71. Zhang HG, Grizzle WE. Exosomes and cancer: a newly described pathway of immune suppression. Clin Cancer Res 2011; 17:959-64; http://dx.doi.org/10.1158/1078-0432.CCR-10-1489; PMID:21224375
72. Clayton A, Mitchell JP, Court J, Linnane S, Mason MD, Tabi Z. Human tumor-derived exosomes down-modulate NKG2D expression. J Immunol 2008; 180:7249-58; PMID:18490724; http://dx.doi.org/10.4049/jimmunol.180.11.7249
73. Viaud S, Terme M, Flament C, Taieb J, André F, Novault S, Escudier B, Robert C, Caillat-Zucman S, Tursz T, et al. Dendritic cell-derived exosomes promote natural killer cell activation and proliferation: a role for NKG2D ligands and IL-15Ralpha. PLoS One 2009; 4:e4942; http://dx.doi.org/10.1371/journal.pone.0004942; PMID:19319200
74. Ashiru O, Boutet P, Fernández-Messina L, Agüera-González S, Skepper JN, Valés-Gómez M, Reyburn HT. Natural killer cell cytotoxicity is suppressed by exposure to the human NKG2D ligand MICA*008 that is shed by tumor cells in exosomes. Cancer Res 2010; 70:481-9; http://dx.doi.org/10.1158/0008-5472.CAN-09-1688; PMID:20068167
75. Ashiru O, López-Cobo S, Fernández-Messina L, Pontes-Quero S, Pandolfi R, Reyburn HT, Valés-Gómez M. A GPI anchor explains the unique biological features of the common NKG2D-ligand allele MICA*008. Biochem J 2013; 454:295-302; http://dx.doi.org/10.1042/BJ20130194; PMID:23772752
76. Xu X, Rao GS, Groh V, Spies T, Gattuso P, Kaufman HL, Plate J, Prinz RA. Major histocompatibility complex class I-related chain A/B (MICA/B) expression in tumor tissue and serum of pancreatic cancer: role of uric acid accumulation in gemcitabine-induced MICA/B expression. BMC Cancer 2011; 11:194; http://dx.doi.org/10.1186/1471-2407-11-194; PMID:21605422
77. Morisaki T, Onishi H, Koya N, Kiyota A, Tanaka H, Umebayashi M, Ogino T, Nagamatsu I, Katano M. Combinatorial cytotoxicity of gemcitabine and cytokine-activated killer cells in hepatocellular carcinoma via the NKG2D-MICA/B system. Anticancer Res 2011; 31:2505-10; PMID:21873167
78. Kohga K, Takehara T, Tatsumi T, Miyagi T, Ishida H, Ohkawa K, Kanto T, Hiramatsu N, Hayashi N. Anticancer chemotherapy inhibits MHC class I-related chain a ectodomain shedding by downregulating ADAM10 expression in hepatocellular carcinoma. Cancer Res 2009; 69:8050-7; http://dx.doi.org/10.1158/0008-5472.CAN-09-0789; PMID:19826051
79. Yamanegi K, Yamane J, Kobayashi K, Ohyama H, Nakasho K, Yamada N, Hata M, Fukunaga S, Futani H, Okamura H, et al. Downregulation of matrix metalloproteinase-9 mRNA by valproic acid plays a role in inhibiting the shedding of MHC class I-related molecules A and B on the surface of human osteosarcoma cells. Oncol Rep 2012; 28:1585-90; http://dx.doi.org/10.3892/or.2012.1981; PMID:22923031
80. Chávez-Blanco A, De la Cruz-Hernández E, Domínguez GI, Rodríguez-Cortez O, Alatorre B, Pérez-Cárdenas E, Chacón-Salinas R, Trejo-Becerril C, Taja-Chayeb L, Trujillo JE, et al. Upregulation of NKG2D ligands and enhanced natural killer cell cytotoxicity by hydralazine and valproate. Int J Oncol 2011; 39:1491-9; http://dx.doi.org/10.3892/ijo.2011.1144; PMID:21805029