Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 3| Issue 1 |JAN-MAR |2013|55-62

Review Article
Pharmaceutical Sciences

OBSTRUCTING SHEDDING OF MIC: THE WAY FORWARD FOR CANCER TREATMENT

D. O. ACHEAMPONG*, J. ZHANG and M. WANG

Department of Molecular Biology, State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China.

Corresponding Author Email: do.acheampong@yahoo.com

ABSTRACT

One of the immunosurveillance mechanisms of the immune system is the expression of Major Histocompatibility Complex class I-related chain molecules A and B (MIC-A and B) on tumor cell surface. MIC-A and B are the ligands of an activating receptor, NKG2D expressed on the natural Killer cells (NK), therefore binding of NK cells to tumor cells through the interaction between NKG2D and MIC-A or MIC-B induces cytolysis of tumor cells. However, clinical observations of most of the human epithelial tumors are found to be MIC-positive rather than MIC-negative, suggesting a functional compromise of the MIC ligand-NKG2D receptor system in cancer patients and therefore allow the growth of MIC positive tumor cells. This is made possible by the release of soluble forms of MIC-A/B from tumor cells which down regulates the NKG2D surface expression on effector cells. This review article therefore sought to discuss the mechanisms underlining the shedding of MIC from tumor cells and how they can be explored by researchers to design drugs for anti-cancer treatment. A literature search on the possible causes of MIC shedding was done. Endoplasmic reticulum protein 5 (ERp5) and A Disintegrin And Metalloproteinase (ADAM10 and ADAM17) have been implicated as responsible for the MIC shedding. Also the α3 ectodomain of the MIC has been identified as target site for these shedding agents. Anti-cancer drugs can possibly be designed using known inhibitors of ERp5, ADAM10 and ADAM17. We also believe, producing therapeutically effective amount of a purified antibody or a polypeptide comprising an antigen-binding fragment thereof that specifically binds to the α3 ectodomain of a MIC polypeptide is the way forward for cancer treatment.

KEY WORDS

Major Histocompatibility Complex class I-related chain molecules A and B, Endoplasmic reticulum protein 5, A Disintegrin and Metalloproteinase, α3 ectodomain, NKG2D, immunosurveillance mechanisms

INTRODUCTION

The interaction between cancer and the immune system is basically characterized by three phases comprising; early immune-mediated tumor elimination, an equilibrium phase and the evasion of tumors from immunosurveillance [1, 2, 3]. As was stated by Waldhauer and his research group [1] scientists face major difficulty in explaining how the immune system is able to recognize malignant autologous cells and to mount an anti tumor immune response in spite of its self-telerant attitude. One of the immunosurveillance mechanisms of the immune system is the expression of MHC class I-related chain molecules A and B (MIC-A and B) on tumor cell surface. MIC-A and B are the ligands of an activating receptor, NKG2D expressed on the natural Killer cells (NK) [4, 5]. Binding of NK cells to tumor cells through the interaction between NKG2D and MIC-A or MIC-B induces cytolysis of tumor cells [4, 5, and 6]. Nevertheless, clinical observations of most of the human epithelial tumors are found to be MIC-positive rather than MIC-negative [7-11]. This therefore suggests a

functional compromise of the MC ligand-NKG2D receptor system in cancer patients and therefore allows the growth of MIC positive tumor cells [11]. In vitro studies elsewhere have shown that engagement of soluble MIC-A/B to NKG2D results in significant reduction in surface NKG2D expression on NK and T cells [10, 11]. This leads to down regulation of NKG2D expression. Shedding of MIC-A/B from tumor cells surface accounts for most of the soluble MIC-A/B [12], therefore any therapeutic prevention of MIC-A/B shedding could lead to the discovery of new cancer treatments. This review article seeks to bring to light the relevance of MIC-A/B to cancer treatment and also some of the methods which have been used by other researchers elsewhere in obstructing the shedding of MIC-A/B as an option for cancer treatment.

MATERIALS AND METHODS

This review was done by compiling references from major databases like PubMed, Science Direct, Google scholar, Scopus, Online journals, Open J Gate, etc.

MIC

MIC-A/B (MHC class I chain-related gene A/B) are transmembrane glycoproteins that function as ligands for human NKG2D. MIC-A and MIC-B are closely related proteins in that, MIC-A shares 85% amino acid identity with MIC-B [4]. The two proteins are distantly related to the MHC class I proteins [4, 5]. They (MIC-A/B) are made up of three extracellular immunoglobulin-like domains but have no capacity to bind peptide or interact with β2-microglobulin [13]. The genes that encode MIC-A/B are found within the major histocompatibity complex (MHC) on human chromosome 6. The MIC-A locus is highly polymorphic with more than 50 recognized human alleles whereas MIC-B locus is polymorphic with a little over 15 recognized human alleles [14, 15]. MIC-A/B is usually expressed minimally on normal cells but is frequently expressed on epithelial tumors and can also be induced by bacterial or viral infections, acting as stress proteins. Receptor for MIC-A/B, NKG2D, is an activating receptor expressed on NK cell, NKT cells, γδ T cells and CD8+ αβ T cells [14, 16]. The recognition of MIC-A/B by the receptor NKG2D results in the activation of cytolytic activity or cytokine production by the effector cells. This recognition is involved in tumor surveillance, viral infections, bacterial infections and autoimmune diseases. Studies elsewhere have shown that, the release of soluble forms of MIC-A/B from tumor cells down regulate the NKG2D surface expression on effector cells resulting in the impairment of the anti-tumor surveillance system [10, 14, and 16].

NKG2D

NKG2D is an activating receptor found on NK cells and CD8+ T cells (αβ and γδ). It was first identified in 1991 and encoded by the *KLRK*1 gene [17]. NKG2D is made up of two disulphide-linked type II transmembrane proteins with short intracellular proteins which are not capable of transducing signals. To overcome this difficulty, they use two adaptor proteins DAP10 and DAP12, which associate a homodimer to the NKG2D and therefore the entire receptor complex appears as hexamer [18]. NKG2D function as an activating receptor was first described in 1999 [19] and its ligands are stressed induced proteins which include MIC-A, MIC-B and ULBP1-6 for human NKG2D, and Rae-1, Mult 1and H-60 for murine NKG2D [20]. These ligands are induced during cellular stress such as infections or genomic stress (cancer), which renders the cell susceptible to NK cell mediated lysis. The function of NKG2D on CD8 T cells is to send co-stimulatory signals to activate them [21]. As cancerous cells are "stressed", NKG2D

ligands become upregulated, rendering the cell susceptible to NK cell-mediated lysis. It is therefore unsurprising that tumor cells have also developed strategies to evade NKG2D responses [18].

Two Protein Agents Implicated for MIC-A/B Shedding

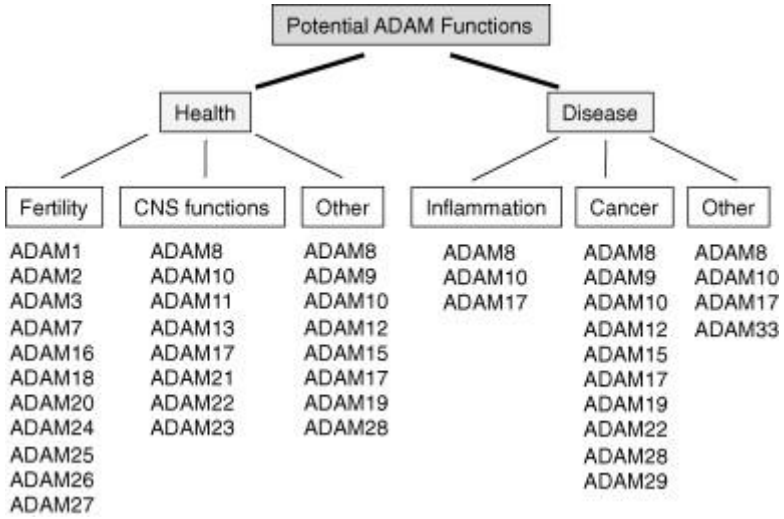
1. ERp5

Endoplasmic reticulum protein 5 (ERp5) is a member of a group of proteins called Endoplasmic reticulum proteins (ERps) which are widely expressed proteins that are associated with Endoplasmic reticulum (ER) and may act as proteases, protein disulfide isomerases, thiol-disulfide oxidases or phospholipases [22]. ERp5, also called PDIA6 (protein disulfide isomerase family A, member 6) or TXNDC7 is a 440 amino acid protein that contains two thioredoxin domains and belong to the protein disulfide isomerase family. It is localized to the melanosome as well as to the lumen of the ER [22]. ERp5 functions as a catalyst to the rearrangement of disulfide bonds in different proteins. Through its catalytic activity, ERp5 is able to reduce the disulfide bond that binds MIC-A/B to tumor cells, thereby releasing MIC-A/B and reducing the rate of tumor expression and down regulating NKG2D receptor [22, 23]. ERp5 specifically binds to the amino acid sequence NGTYQT located in the α3 ectodomain of a MIC polypeptide therefore any therapeutic intervention should be targeted to this site to avoid ERp5 attachment which leads to the shedding of the MIC [24, 25].

2. ADAM

ADAM (A Disintegrin And Metalloproteinase) is a family of peptidase proteins [26, 27]. It is also referred to as the adamalysin family or MDC family (metalloproteinase-like, Disintegrin-like, Cysteine rich) [26, 28]. They are classified as sheddases because they are able to cut off extracellular portions of transmembrane proteins [29]. For example, according to Inja Waldhauer and his research group, ADAM10 and ADAM17 which are members of the ADAM's family are associated with MIC-A shedding by tumor cells that promote tumor growth [30]. Therefore therapeutic ADAM inhibitors can potentially be anti-cancer therapy [30]. **Fig. 1** illustrates the potential functions of the family members of ADAM.

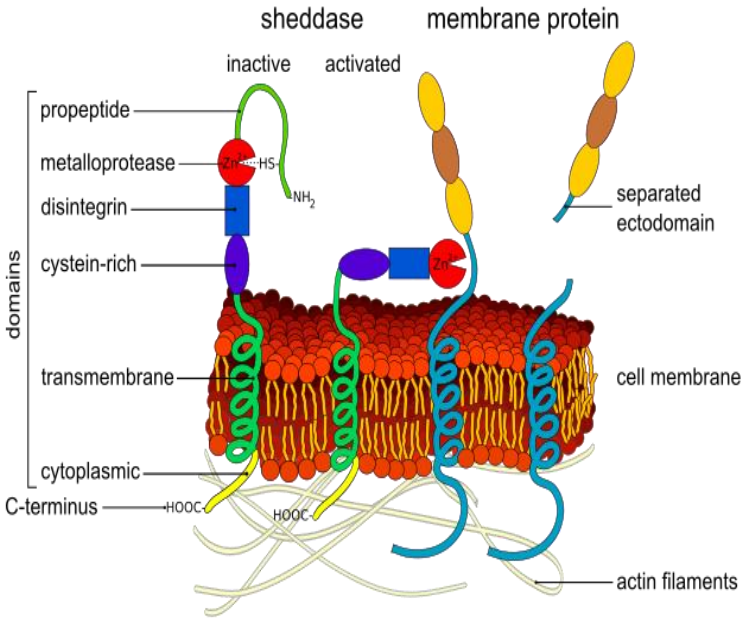
Fig. 1 Health and Disease Conditions Associated with ADAM members [31]



I. ADAM10

ADAM10 (A Disintegrin and metalloproteinase domain-containing protein10) is a protein encoded by the ADAM10 gene in human. It is known to involve in ectodomain shedding of various cell surface proteins such as growth factors, receptor and their ligands, cytokines and cell adhesion molecules [31]. As was reported by Inja Waldhauer and his group, it could play a role in the MIC-A shedding by tumor cells [30]. Although the exact mechanism of ADAM10 has not been thoroughly investigated, its active site is homologous to those of well studied zinc-proteases such as carboxypeptidase. Therefore it is believed that ADAM10 utilizes a similar mechanism as these enzymes. As shown in **Fig. 2**, in zinc proteases, the key catalytic elements have been identified as a glutamate residue and a Zn^{2+} ion coordinated to histidine residue [32].

Fig. 2 Diagram of an ectodomain shedding ADAM metalloprotease[33].



II. ADAM17

ADAM metallopeptidase domain 17 (ADAM17), also called TACE (tumor necrosis factor- α-converting enzyme), is a 70-KDa enzyme [34]. It is known to be involved in the processing of tumor necrosis factor alpha at the surface of the cell. This process which is also known as shedding, involves the cleavage and release of a soluble ectodomain from membrane-bound pro-proteins [30, 34]. Share most of the features of ADAM10 described above [30].

Proposed Therapeutic interventions to the Shedding of MIC from Tumor cells

As already mentioned, MIC shedding is considered a principal mechanism of tumor cells to escape from NKG2D mediated immunosurveillance in human. This does not only lead to reduction of MIC surface density but also generates soluble MIC, which eventually down regulate NKG2D and therefore results in immunosuppression [30, 35, 36]. This constituted the reason for the research by Inja Waldhauer and his group [30]. They came out with the following results after their research; (a) MICA cleavage occurs at the surface of tumor

cells, (b) MICA is cleaved within the juxta membranous stalk, (c) MICA cleavage is dependent on the length but not on the sequence of the stalk, (d) MICA shedding is inhibited by broad-range metalloproteinase inhibitors but not by (Matrix metalloproteinase) MMP-specific Tissue inhibitor of metalloproteinase 2 (TIMP2), (e) MICA shedding is induced by protein kinase C (PKC), (f) constitutive and induced MICA shedding is variably affected by ADAM-specific inhibitors GI254023X and GW280264X, and (g) shedding of MICA (and ULBP2) is suppressed on silencing of ADAM10 and/or ADAM17. They were however quick to state that, there is a possibility that other proteases additionally contribute to MIC-A shedding [30]. In another related research by Hermann C Altmeppen and his group on "Lack of a-disintegrin-and-metalloproteinase ADAM10 leads to intracellular accumulation and loss of shedding of the cellular prion protein in vivo", their findings supported the fact that ADAM10 could play a role in the shedding of many proteins [37]. They used neuron-specific ADAM10 knockout mice to show that ADAM10 is the sheddase of PrP^c and that its absence in vivo leads to increased amounts and accumulation of PrP^c in the early secretory pathway by affecting its posttranslational processing [37]. All things being equal, application of appropriate ADAM inhibitors could obstruct the shedding of MIC from tumor cells. This could be explored further to develop treatment for cancer.

Jennifer D. Wu worked on the topic "Methods of treating cancer by inhibiting MIC shedding" and subsequently received patent for her findings [38]. Her invention was based on the fact that expression of murine NKG2D ligands on tumor cells has been shown to be effective in activating NK-mediated tumor elimination experimentally [39]. Her invention describes methods for treating cancer comprising administering a therapeutically effective amount of an agent that prevents MIC shedding mediated by the alpha-3 ecto domain of MIC of a tumor cell, thus rendering the tumor cell more sensitive to innate immune cell rejection [38]. One of the highlights of her invention was the proposed use of a therapeutically effective amount of a purified antibody or a polypeptide comprising an antigen-binding fragment thereof that specifically binds to the amino acid sequence NGTYQT (SEQ ID NO: 1) located in the α3 ectodomain of a MIC polypeptide; wherein the interaction of the MIC polypeptide and ERp5 is inhibited; and whereby the shedding of said MIC polypeptide is inhibited; whereby said MIC-positive cancer is treated [38]. This demonstrates clearly that ERp5 has something to do with the shedding of MIC from tumor cells and it does it by binding to α3 ectodomain of a MIC polypeptide. Therefore any therapeutic intervention that can bind to this specific site or prevent ERp5 from binding to this site as has been discovered by Jennifer D. Wu could prove effective for treating cancer. Another research by Jennifer D. Wu and her research team entitled "Obstructing Shedding of the Immunostimulatory MHC Class I Chain-Related Gene B Prevents Tumor Formation" actually identified the α3 ectodomain of MIC as the target site for shedding. After extensive research and study on the subject matter, they reported that partially replacing the α3 domain of MICB protects from tumor cell shedding. This was after they have generated mutant form MICB.A2 by replacing part of the α3 ectodomain of MICB (amino acids 215-274) with the corresponding residues from HLA-A2 and using ELISA assay to assess the degree of shedding, knowing very well that NKG2D only interacts with the α1 and α2 domain of MIC [11].

In another research entitled MHC Class I chain-related protein A antibodies and shedding are

associated with the progression of multiple myeloma, Masahisa Jinushi and his research team made a very striking conclusion which implicated ERp5 as the cause of MIC shedding from the tumor cell they worked on [40]. They observed that, during full-blown multiple myeloma (MM), the up-regulation of ERp5 promotes efficient MICA shedding, which evokes NKG2D internalization and immune suppression. Thus, stage-specific alterations in MICA activity are associated with the conversion of immune equilibrium to immune escape [40].

In another development, Koji Yamanegi and his group's quest to search for the remedy to the shedding of MIC from tumor cells led them to investigate the effect of sodium valproate (VPA), a histone deacetylase inhibitor, on the production of cell-surface and soluble MIC and NK cell-mediated cytotoxicity in four human osteosarcoma cells [6]. Their result showed that, VPA at 0.5 and 1.0 mM induced acetylation of histones bound to MICA and B gene promoters, increased cell-surface but not soluble MIC, and therefore augmented the susceptibility of osteosarcoma cells to NK cell-mediated cytotoxicity. They therefore concluded that VPA in combination with immunotherapy activating cytotoxic immune cells could be useful to treat osteosarcomas [6]. This should be investigated further since it could lead to the discovery of an anti-cancer treatment.

CONCLUSION

MIC shedding is considered a principal mechanism through which tumor cells escape from NKG2D-mediated immune surveillance in human [10, 11]. The shedding of MIC by tumor cells can therefore promote tumor growth. Considering the fact that MIC is a stress protein mostly expressed on stressed cells like tumor cells, it could have been easier to eliminate tumor cell through NKG2D-mediated immunosurveillance but for its shedding tendencies. To overcome this difficulty, researchers have tried understanding the mechanism of MIC shedding through research and have implicated ERp5, ADAM10 and ADAM17 as the likely cause [22, 30]. Also the α3 ectodomain of the MIC has been identified as target site for the shedding agents [11]. Jennifer D. Wu has even gone further to identify the specific site the enzymes responsible for the shedding attaches [38]. We believe that armed with all this relevant information about MIC shedding, it is time researchers explored it to come out with efficient cancer treatment drugs, as it has been done by Jennifer D. Wu. Anti-cancer drugs can possibly be designed using known inhibitors of ERp5, ADAM10 and ADAM17. We also believe, producing therapeutically effective amount of a purified antibody or a polypeptide comprising an antigen-binding fragment thereof that specifically binds to the amino acid sequence NGTYQT (SEQ ID NO: 1) located in the α3 ectodomain of a MIC polypeptide as prescribed by Jennifer D. Wu in her patent report is the way forward for cancer treatment [38].

REFERENCE

[1] Waldhauer I, *et al*: Tumor-Associated MICA is shed by ADAM Proteases. *Cancer Res*, 68(15): 6368-6376, (2008).

[2] Dunn GP, Old LJ and Schreiber RD: The three Es of cancer immunoediting. *Annu Rev Immunol*, 22: 329-360, (2004).

[3] Smyth MJ, Dunn GP and Schreiber RD: Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Adv Immunol* , 90: 1-50, (2006).

[4] Waldhauer I and Steinle A: NK cells and cancer immunosurveillance. *Oncogene,* 27: 5932-5958, (2008).

[5] Nausch N. and Cerwenka A: NKG2D ligands in tumor immunity. *Oncogene*, 27: 5944-5958, (2008).

[6] Yamanegi K, *et al*: Sodium valproate, a histone deacetylase inhibitor, augments the expression of cell-surface NKG2D ligands, MICA/B, without increasing their soluble forms to enhance susceptibility of human osteosarcoma cells to NK cell-mediated cytotoxicity. Oncology Reports, 24: 1621-1627, (2010).

[7] Groh V, Rhinehart R, Secrist H, Bauer S, Grabstein KH and Spies T: Broad tumor-associated expression and recognition by tumor-derived gy Tcel ls of MICA and MICB. Proc Natl Ac ad Sci USA, 96:6879-6884, (1999).

[8] Vetter CS, GrohV, thor Straten P, SpiesT, Brocker EB and Becker JC: Expression of stress-induced MHC class I related chain molecules on human melanoma. J Invest Dermatol, 118: 600-605, (20 02).

[9] Jinushi M, Takehara T, Tatsumi T, *et al*: Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. Int J Cancer, 104:354-361, (2003).

[10] Wu JD, Higgins LM, Steinle A, Cosman D, Haugk K, Plymate SR: Prevalent expression of the immunostimulatory MHC class I chain-related molecule is Counteracted by shedding in prostate cancer. J Clin Invest, 114:560-568, (2004).

[11] Wu JD *et al*: Obstructing Shedding of the ImmunostimulatoryMHC Class I Chain-Related Gene B PreventsTumor Formation. Clin Cancer Res, 15(2): 632-640 2009.

[12] Marten A, von Lilienfeld-Toal M, Buchler MW, Schmidt J: Soluble MIC is elevated in the serum of patients with pancreatic carcinoma diminishing (γδ) T cellcytotoxicity. Int J Cancer, 119:2359-2365, (2006).

[13] Trowsdale J, Lee J, Kelly A, *et al*: Isolation and sequencing of a cDNA clone for a human HLAABC antigen. Mol. Biol. Med, 2 (1): 53–61, (1984).

[14] Marsh SG *et al*: Nomenclature for factors of the HLA System. Tissue antigens, 65: 301–369, (2005).

[15] Brown MA, Crane AM and Wordsworth BP: Genetic aspects of susceptibility, severity, and clinical expression in ankylosing spondylitis. Curr Opin Rheumatol, 14(4): 354–60, (2002).

[16] Carrington M, O'Brien SJ: The influence of HLA genotype on AIDS. Annu Rev Med, 54: 535–551, (2003).

[17] Houchins J, *et al*: 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, (1991).

[18] Zafirova B *et al*: Regulation of immune cell function and differentiation by the NKG2D receptor. Cell Mol Life Sci, 68 (21): 3519–3529, (2011).

[19] Bauer S *et al.*: Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science, 285 (5428): 727–729, (1999).

[20] Raulet DH: Roles of the NKG2D immunoreceptor and its ligands. Nature Reviews Immunology, 3: 781-790, (2003).

[21] González S *et al*: NKG2D ligands: key targets of the immune response. Trends in Immunology, 29 (8), (2008).

[22] Kaiser B.K., et al: Disulphide-isomerase-enabled shedding of tumour-associated NKG2D ligands. Nature, 447: 482-486, (2007).

[23] Jordan PA, *et al*: A role for the thiol isomerase protein ERp5 in platelet function. Blood, 105: 1500-1507, (2005).

[24] Wu JD**,** ***et al***: Prevalent Expression of the Immunostimulatory MHC Class I Chain-related Molecule is Counteracted by Shedding in Prostate Cancer. J Clin Invest, 114: 560-568, (2004).

[25] Salih, *et al*:Down-Regulation of MICA on Human Tumors by Proteolytic Shedding. J Immunol, 169: 4098-102, (2002).

[26] Edwards DR, Handsley MM and Pennington CJ: The ADAM etalloproteinases. Mol. Aspects Med, 29 (5): 258–289, (2008).

[27] Brocker C, Vasiliou V and Nebert DW: Evolutionary divergence and functions of the ADAM and ADAMTS gene families. Human Genomics, 4 (1): 43-55, (2009).

[28] Wolfsberg TG, Straight PD, Gerena RL, *et al*: ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. Dev. Biol. 169 (1): 378–383, (1995).

[29] Liu PC, *et al*: Identification of ADAM10 as a major source of HER2 ectodomain sheddase activity in HER2 overexpressing breast cancer cells. Cancer Biology and Therapy 5 (6): 657–664, (2006).

[30] Waldhauer I, *et al*: Tumor-Associated MICA Is Shed by ADAM Proteases. Cancer Res, 68(15): 6368-6376, (2008).

[31] Reiss K and Saftig P. The A Disintegrin and Metalloprotease (ADAM) family of sheddases: Physiological and cellular functions. Seminar in Cell and Developmental Biology, 20(2):126-137, (2009).

[32] Lolis E and Petsko GA:Transition-state analogues in protein crystallography: probes of the structural source of enzyme catalysis. Annual Review of Biochemistry, 59: 597–630, (1990).

[33] Ectodomain shedding en.svg

[34] Moss ML, Jin SL, Milla ME *et al*: Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha. Nature 385 (6618): 733–36, (1997).

[35] Salih HR, Antropius H, Gieseke F, et al: Functional expression and release of ligands for the activating

immunoreceptor NKG2D in leukemia. Blood, 102: 1389-1396, 2003.

[36] Groh V, Wu J, Yee C, and Spies T: Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature, 419:734–738, (2002).

[37] Altmeppen CH, *et al*: Molecular Neurodegeneration. Lack of a-disintegrin-and-metalloproteinase ADAM10 leads to intracellular accumulation and loss of shedding of the cellular prion protein in vivo. Molecular Neurodegeneration , 6(36) :1-12, (2011).

[38] Wu JD: Methods for treating cancer by inhibiting MIC shedding. United State Patent Wu. US 8,182,809 B1. May 22, (2012).

[39] Cerwenka A, *et al*: Retinoic Acid Early Inducible Gene Define a Ligand Family for the Activating NKG2D Receptor in Mice. Immunity. 12: 721-727, (2000).

[40] Jinushi M, *et al*: MHC class I chain-related protein A antibodies and shedding are associated with the progression of multiple myeloma. PNAS, 105(4):1285–1290, (2008).

***Corresponding Author:**
Desmond Omane Acheampong,
Department of Molecular Biology,
State Key Laboratory of Natural Medicines,
China Pharmaceutical University,
Nanjing 210009, China.
Email: do.acheampong@yahoo.com