

# How to exploit stress-related immunity against Hodgkin's lymphoma

## Targeting ERp5 and ADAM sheddases

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**Abbreviations:** ADAM, a disintegrin and metalloproteinase; ATRA, all-*trans* retinoic acid; ERp5, endoplasmic reticulum protein 5; HDAC, histone deacetylase; IFN, interferon; IL, interleukin; LN, lymph node; LNMSC, LN mesenchymal stromal cell; mAb, monoclonal antibody; MIC, MHC Class I polypeptide-related sequence; MMP, matrix metalloproteinase; MSC, mesenchymal stromal cell; N-BPs, aminobisphosphonates; P-Ag, phosphoantigen; PDI, protein disulfide isomerase; RIP, regulated intramembrane proteolysis; RS, Reed-Stenberg; TGFβ1, transforming growth factor β1; TNFα, tumor necrosis factor α; ULBP, UL16-binding protein; VPA, valproic acid

Stress-related immunity can be activated in the course of lymphoproliferative disorders, including Hodgkin's lymphoma, upon the interaction between killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D) on effector lymphocytes and NKG2D ligands (NKG2DL), such as MHC class I polypeptide-related sequence A (MICA), MICB and various UL16-binding proteins (ULBPs), on lymphoma cells. However, NKG2DLs can also bind NKG2D upon shedding, thus affecting the recognition of lymphoma cells by the immune system. The proteolytic cleavage of MICA depends on protein disulfide isomerase family A, member 6 (PDIA6, a thiol isomerase best known as ERp5) as well as on the disintegrins and metalloproteinases ADAM metallopeptidase domain 10 (ADAM10) and ADAM17, which also cleave ULBPs. These enzymes can be targeted in novel therapeutic schemes to avoid the escape of malignant cells from stress-evoked immune responses.

### Introduction

The incidence of lymphoproliferative disorders, including Hodgkin's lymphoma, in the Western world is increasing.<sup>1-4</sup> Although most of these patients can be cured with modern treatment strategies, almost one third of them die due to relapsing or progressive disease. Indeed, several lymphomas become resistant to conventional therapies, and non-responding patients often do not benefit from alternative therapeutic schemes.

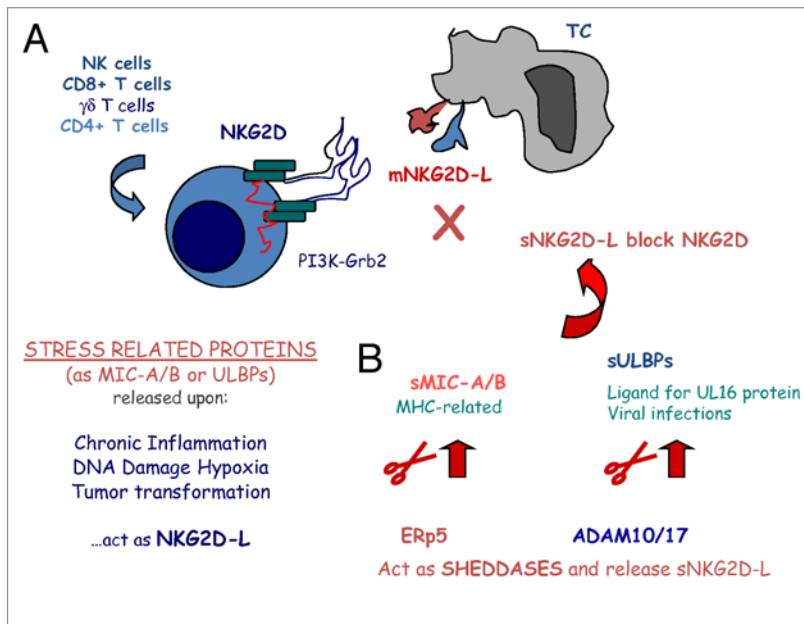
This casts interest on the development of new diagnostic and therapeutic tools.<sup>1-4</sup> Recent preclinical and clinical studies have demonstrated that the tumor microenvironment, and in particular the interface between malignant and immunoreactive cells, represents a promising therapeutic target.<sup>1-4</sup> Previous data from our group confirm this hypothesis in the setting of Hodgkin's as well as non-Hodgkin's lymphoma.<sup>5,6</sup> Herein, we describe the immune response elicited by lymphoproliferative disorders from the standpoint of the so-called "stress-related immunity," a mechanism of preservation of tissue homeostasis that might constitute a target for therapeutic interventions.

### Hodgkin's Lymphoma

Hodgkin's lymphoma was first described as a sort lymphogranulomatosis and was then recognized to be a neoplasm derived from B lymphocytes. Hodgkin's lymphoma accounts for 11% of all lymphomas and is the most common subtype of lymphoma in the young. About 20% of Hodgkin's lymphoma patients are intrinsically resistant to conventional therapeutic regimens or rapidly become so. The typical histological features of Hodgkin's lymphoma are CD20<sup>+</sup> Reed-Sternberg (RS) cells for the classical variant of the disease, and the so-called "lymphocyte-predominant cells" in the subtype of disease known as nodular lymphocyte predominant Hodgkin's lymphoma. Of note, in Hodgkin's lymphoma immunoreactive cells outnumber their malignant counterparts, suggesting that an abnormal or insufficient immune response is one of the co-factors of oncogenesis and/or tumor progression. The release of cytokines, enzymes and growth factors may alter the function of non-transformed cells of the tumor microenvironment, including mesenchymal stromal cells and macrophages, as well as their physical interactions with each other and with cancer cells. In this context, RS cells may originate from the chronic inflammatory state that accompanies inefficient immune

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**Figure 1.** Stress-related immunity. (A) Stress molecules such as MHC class I polypeptide-related sequence A (MICA), MICB and various UL16-binding proteins (ULBPs), which are collectively known as NKG2D ligands (NKG2DLs), are expressed at the surface of epithelial or cancer cell upon chronic inflammation, infection or oncogenic transformation. NKG2DLs directly stimulate natural killer (NK) cells or unconventional T cells, including  $\gamma\delta$  T cells, through NKG2D receptors to trigger antitumor immune responses. (B) Enzymes involved in the cleavage and shedding of NKG2DLs, i.e., the disulfide isomerase ERp5, ADAM metallopeptidase domain 10 (ADAM10) and ADAM17.

responses. In turn, RS cells themselves contribute to the establishment of a microenvironment that favors the growth of cancer cells by suppressing antitumor immunity.<sup>1-4</sup>

### Stress-related Immunity in Cancer Immunosurveillance

In the last years the concept of “lymphoid stress surveillance” has emerged (Fig. 1).<sup>7,8</sup> According to this concept, the immune system is equipped with 2 types of responses: (1) the classic antigen-specific immune response, elicited by myeloid antigen-presenting cells and resulting in the expansion of antigen-specific T and B lymphocytes; (2) a response to stress signals mediated by so-called “unconventional T lymphocytes” independently from antigen-presenting cells. The activation of myeloid cells such as neutrophils and macrophages is rapid but relatively untargeted (innate immunity). Conversely, antigen-specific immune responses are precisely targeted (adaptive immunity), but need time to develop. Stress-elicited responses such as those mediated by unconventional T lymphocytes take place with a kinetics that stays in between the rapid activation of myeloid cells and the elicitation of antigen-specific immunity. In this scenario, infected or injured cells (be they epithelial or parenchymal) express (or upregulate) a set of molecules on their surface as a signature of cell damage. These molecules are commonly referred to as stress-induced antigens. Besides the urate and ATP, which are released from dying cells,<sup>9</sup> many stress antigens including MHC class I

polypeptide-related sequence A (MICA), MICB and various UL16-binding proteins (ULBPs) may not be recognized by myeloid cells. Rather, these molecules are recognized by natural killer (NK) cells, CD8<sup>+</sup> memory T cells, and a broad set of unconventional T lymphocytes, of which  $\gamma\delta$  T cells are the prototype.<sup>5-13</sup> Although not sufficient to clear the body from a specific pathogen or prevent oncogenesis, lymphoid stress surveillance can inhibit the dissemination of infected or malignant cells, maintain tissue integrity, and regulate multiple adaptive responses, as discussed below.

### MICA, MICB and ULBPs as stress molecules and NKG2D ligands

It is now clear that also in the course of malignant transformation stress signals such as MICA, MICB, and ULBPs can be exposed on the membrane of stressed cells.<sup>9-12</sup> These molecules are able to bind killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D) expressed by cytolytic T and NK cells, and are cumulatively referred to as NKG2D ligands (NKG2DLs). Upon engagement by one of these ligands, NKG2D delivers an activating signal that results in the release of lytic enzymes, such as perforin or granzymes, and antitumor cytokines like tumor necrosis factor  $\alpha$  (TNF) $\alpha$  (Fig. 1).<sup>9-14</sup> As mentioned above, NKG2DLs encompass MICA and MICB, which can be upregulated at the surface of epithelial cells by physicochemical perturbations and inflammation, and ULBP1-6, which are also ligands of the UL16 protein produced by cytomegalovirus-infected cells.<sup>8-12</sup> All these molecules can be naturally expressed at the surface of cells in the course of oncogenic transformation (including lymphomagenesis). In addition, the upregulation of these molecules at the surface of malignant cells (including lymphoma cells) can be triggered in vitro and in vivo by specific drugs including all-*trans* retinoic acid (ATRA) and valproic acid (VPA).<sup>14-16</sup> Of note, MICA, MICB, and ULBPs can be cleaved (and hence released in the extracellular milieu) by protein disulfide isomerase family A, member 6 (PDIA6, a thiol isomerase best known as ERp5) as well as by ADAM metallopeptidase domain 10 (ADAM10) and ADAM17.<sup>14,17,18</sup> Soluble NKG2DLs bind to NKG2D but are unable to deliver activating signals (see below).

### NKG2D in $\gamma\delta$ T effector cell function

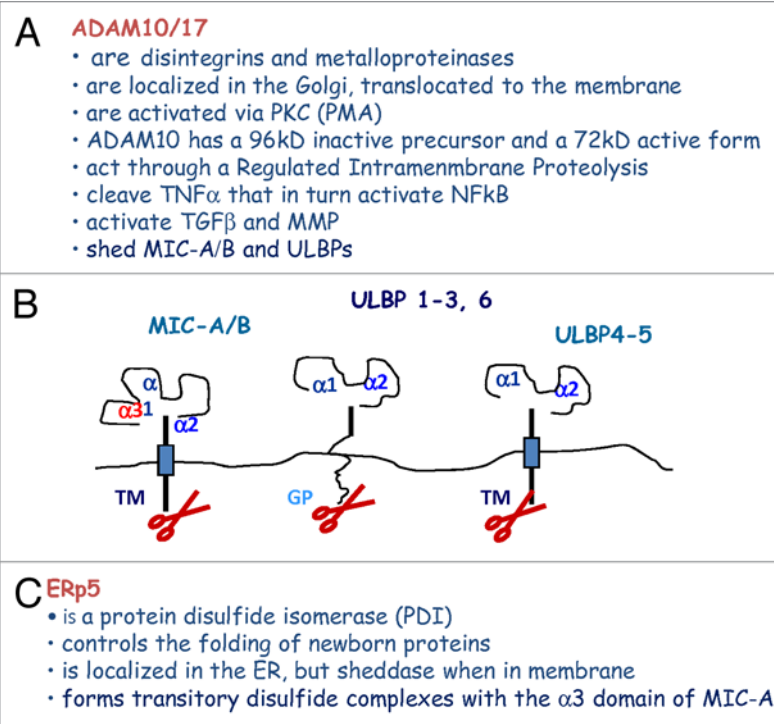
Together with other unconventional T lymphocytes,  $\gamma\delta$  T cells are prominent mediators of stress-related immune responses.<sup>7-9</sup> T lymphocytes bearing the  $\gamma\delta$  T-cell receptor (TCR) represent a relevant proportion of the mucosal-associated lymphoid tissue, which is known to play an important role in the first line of defense against viral, bacterial and fungal pathogens. Two main subsets of  $\gamma\delta$  T cells are known: circulating V $\delta$ 2 T lymphocytes are involved in the response to mycobacteria, the Epstein-Barr virus and some solid tumors, while V $\delta$ 1 T cells, which reside in mucosal-associated lymphoid tissues, contribute to the control of *Listeria monocytogenes*, cytomegalovirus and certain

hematological malignancies.<sup>5,6,12,15,16,19,20</sup> Both these  $\gamma\delta$  T-cell subsets can interact with stress-induced MICA, MICB, and ULBPs, mainly through NKG2D receptors. In  $\gamma\delta$  T cells, it is a TCR-associated variant of NKG2D that appears to bind these stress molecules. Just upon the engagement of NKG2D, an activating signal is delivered to  $\gamma\delta$  T lymphocytes, which hence proliferate, produce pro-inflammatory and antimicrobial cytokines, such as interferon  $\gamma$  (IFN) $\gamma$  or TNF $\alpha$ , and release lytic enzymes to destroy bacteria or infected cells.<sup>9-11</sup> A similar mechanism is harnessed by  $\gamma\delta$  T lymphocytes that are confronted to transformed cells that overexpress NKG2DLs as a result of oncogenic transformation.<sup>5-10</sup> Another potent stimulus for  $\gamma\delta$  T cells of the V $\delta$ 2 subset, which operates through the  $\gamma\delta$  TCR, is represented by low molecular weight phosphoantigens (P-Ags).<sup>21-25</sup> Consistent with the stress surveillance model, P-Ags can be autologous, such as isoprenylpyrophosphate (which accumulates in many virus-infected or transformed cells), or microbial, such as (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate, a metabolic intermediate specific of many prokaryotes and parasites.<sup>21-24</sup> Of clinical interest, aminobisphosphonates (N-BPs), which are widely prescribed for the treatment of osteoporosis and some malignancies, indirectly activate V $\gamma$ 9V $\delta$ 2 cells as they inhibit farnesyl pyrophosphate synthase and hence favor the accumulation of pyrophosphates that act as P-Ags.<sup>25</sup>

#### Sheddases and disulfide isomerases: physiopathological role

Sheddases are membrane-bound enzymes that cleave the extracellular portion of transmembrane proteins, releasing a soluble form of their ectodomains from the cell surface. Some sheddases are membrane proteins themselves that belong to the matrix metalloproteinase (MMP) or aspartic protease family. The activity of sheddases can be constitutive or regulated by various determinants, including the activation of protein kinase C, Ca<sup>2+</sup> influx, and lipid rafts.<sup>26-28</sup> A single sheddase may cleave a variety of substrates. For instance, ADAM17, which was initially identified as TNF $\alpha$ -converting enzyme (TACE), is known to shed a variety of growth factors, receptors and adhesion molecules.<sup>26-29</sup> This suggests that the overall conformation of the substrates is more important than primary amino acid sequences in determining the susceptibility to cleavage by sheddases. Along similar lines, multiple sheddases can cleave the same substrate. For instance, ADAM17, ADAM10, and MMP14 (also known as MT1-MMP) are all known to shed CD44, an adhesion molecule that interacts with hyaluronic acid in the extracellular matrix.

The juxtamembrane cleavage of transmembrane proteins resulting in the release of their cytoplasmic domain, which can be further processed to generate additional proteolytic fragments, has been termed regulated intramembrane proteolysis (RIP). Sheddases can either up- or downregulate the activity of their substrates. Shedding may indeed abrogate the function of a protein or result in the generation of fragments that transduce a

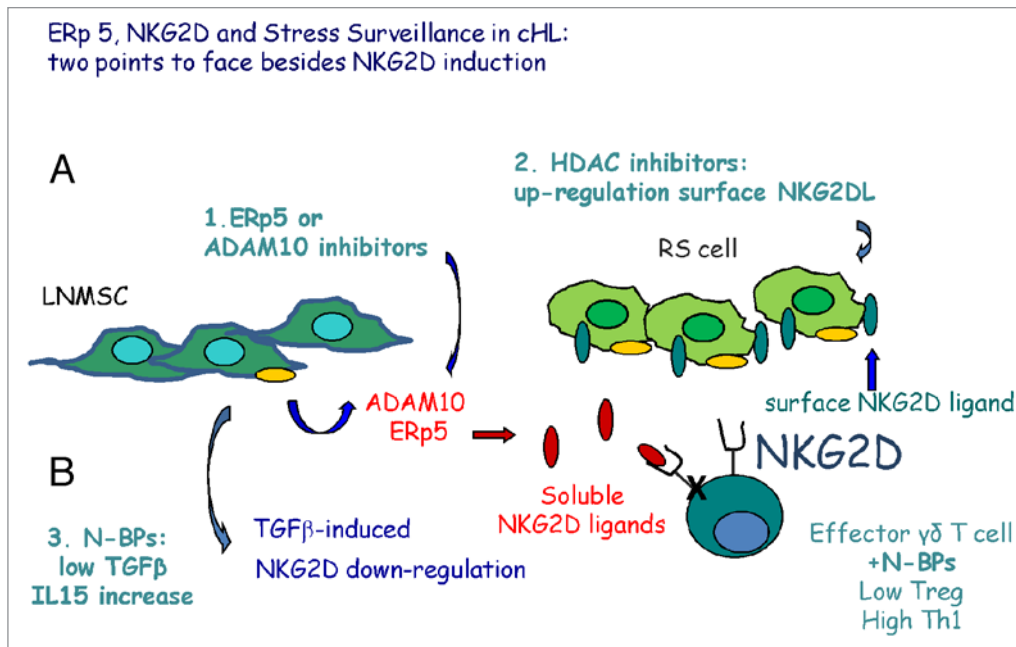


**Figure 2.** Enzymes involved in the cleavage and shedding of NKG2D ligands. (A) Characteristics, functions and substrates of ADAM metalloproteinase domain 10 (ADAM10) and ADAM17. (B) NKG2D ligands (NKG2DLs) such as MHC class I polypeptide-related sequence A (MICA), MICB and various UL16-binding proteins (ULBPs) can be cleaved by ADAMs and ERp5. (C) Substrates, characteristics and function of ERp5.

signal in conjunction with RIP (Fig. 2). For instance, the soluble variant of several cytokine receptors, including interleukin (IL)-15 receptor (IL-15R), competes with its membrane-bound counterparts, while others, such as soluble IL-6R, exert an agonistic activity when bound to their ligands.<sup>26,27</sup>

Sheddases can regulate cell adhesion and migration as well. For example, CD44-dependent migration results from the ADAM17-mediated shedding of the CD44 ectodomain at the leading edge of the cell. The extension of the lamellipods triggers an influx of Ca<sup>2+</sup> ions in the cytoplasm, and CD44 shedding by Ca<sup>2+</sup>-activated ADAM10 at the trailing edge facilitates cell detachment. Concomitantly, RIP creates a cytoplasmic CD44 fragment that promotes the neosynthesis of CD44. Such a link between the proteolysis and neosynthesis of CD44 results in its rapid turnover, which ensures an efficient migratory response.<sup>29</sup>

Unlike sheddases, protein disulfide isomerases (PDIs) are localized in the endoplasmic reticulum of eukaryotic cells, catalyzing the formation and breakage of disulfide bonds between cysteine residues as proteins fold.<sup>30,31</sup> The activity of PDIs allows proteins to quickly assume the arrangement of disulfide bonds that characterize their fully folded state. Thus, PDIs de facto catalyze protein folding. PDIs contain 4 thioredoxin-like domains, 2 of them retaining the canonical CXXC motif. The reduced (that is, dithiol-containing) form of PDIs is able to catalyze the reduction of mispaired thiol residues of a particular substrate, acting as an isomerase.<sup>32</sup> Therefore, PDIs catalyze the



**Figure 3.** Possible therapeutic interventions to promote stress-related immune responses within the lymphoma microenvironment. **(A)** Influencing the effects of soluble NKG2D ligands (NGK2DLs): (1) ERp5 and ADAMs inhibitors can limit the shedding of NKG2DLs; (2) histone deacetylase (HDAC) inhibitors such as valproic acid (VPA) can stimulate the exposure of NKG2DLs on the cell surface. **(B)** Acting on immunoregulatory cytokines produced by the microenvironment: (3) the administration of aminobisphosphonates (N-BPs) to inhibit transforming growth factor  $\beta$  1 (TGF $\beta$ 1) production by lymph node mesenchymal stromal cells (LNMSCs), stimulating the generation of effector memory ( $T_{EM}$ )  $\gamma\delta$  T cells ( $T_H$ 1 response) rather than regulatory T cells (Tregs). Also, N-BPs can stimulate LNMSCs to secrete increased amounts of interleukin-15 (IL-15), which promotes NKG2D expression by lymphocytes.

posttranslational modification known as disulfide exchange. Such exchange reactions can occur intramolecularly, leading to the rearrangement of disulfide bonds within a single protein.<sup>33</sup> Another major function of PDIs relates to their activity as chaperones, aiding misfolded proteins to reach a correctly folded state without the aid of enzymatic disulfide shuffling. PDIs assist in the loading of peptides onto MHC class I molecules, allowing the presentation of antigen determinant by antigen-presenting cells to T lymphocytes. PDIs also break bonds in the HIV-1 protein gp120 as the virus infects CD4<sup>+</sup> cells, and their presence is required for HIV-1 to infect lymphocytes and monocytes. Recent evidence supports an alternative role for this family of proteins on the cell surface as they can be involved in receptor remodeling and recognition.<sup>30,33</sup>

### ADAMs and Erp5

#### ADAMs

ADAMs (an acronym that stands for “a disintegrin and metalloproteinase”) are cell surface enzymes involved in cell adhesion and proteolysis. There are 21 functional members of the human ADAM family, but only 13 of them are active metalloproteinases, as the others lack an essential Zn-binding motif in the catalytic site. A prototypical ADAM comprises an N-terminal pro-domain that maintains the enzyme in a latent state, followed by metalloproteinase, disintegrin, cysteine-rich, and EGF-like domains, a transmembrane region and a cytoplasmic tail of variable length.<sup>26,34,35</sup> Structural analyses

revealed that ADAMs assume a C-shaped structure with the metalloproteinase and cysteine-rich domains at the top and bottom of the C. In this configuration, the disintegrin domain is found within the C-shaped structure. Such a “disintegrin loop” has been previously proposed as the domain that mediates the interaction between ADAMs and integrins. In contrast, the cysteine-rich domain, containing a hypervariable region that is specific for each ADAM, constitute the sideward-facing part of the C. This domain might allow the docking of potential ADAM substrates in the proximity of the cell membrane. For ADAMs to be catalytically active, their pro-domain must be cleaved. This can be stimulated by redox changes in the sulfhydryl groups of the disintegrin/cysteine-rich region, an activation process also known as “cysteine-switch.” In the case of ADAM10, the removal of the pro-domain is mediated by the pro-protein convertases furin and/or proprotein convertase subtilisin/kexin type 7 (PCSK7) in the Golgi compartment.<sup>34,35</sup> In addition, the cytoplasmic tail of some ADAMs, such as ADAM17, contains binding sites for SRC homology 3 (SH3) domain-containing proteins, as well as serine, threonine, and tyrosine residues that are potential sites for phosphorylation. Proteolytically active ADAMs act as ectodomain sheddases. In particular, ADAM10 catalyzes the initial cleavage of molecules that are then subjected to RIP, while ADAM17 directly promotes the release of the ectodomain of its substrates. For instance, ADAM17 activates TNF $\alpha$  by releasing the active form of the cytokine from the pro-TNF $\alpha$  precursor. Both ADAM10 and ADAM17 appear to be activated in several solid tumors and can cleave MICA as well as ULBPs to release



them in a soluble form (Fig. 2).<sup>6,17,18</sup> Recently, we have reported that ADAM10 is overexpressed in the Hodgkin's lymphoma microenvironment, at the level of both RS and mesenchymal stromal cells, participating in the shedding of NKG2DLs from neoplastic cells.<sup>6</sup>

### ERp5

ERp5 is a thiol isomerase of 440 residues that contains 2 thioredoxin domains. ERp5 is localized to the lumen of the endoplasmic reticulum, where it catalyzes the rearrangement of disulfide bonds in a variety of different proteins, thus contributing to their proper folding (Fig. 2). Thiol isomerases are required for protein maturation, speeding up a process that may take several days into one that lasts less than an hour (which is the time needed for the secretion of nascent proteins). Alongside, thiol isomerases prevent the accumulation of misfolded proteins, a which is involved in a number of diseases.<sup>30,31</sup> It is becoming evident that thiol isomerases are not confined to the ER but rather catalyze the rearrangement of disulfide bond also at extrareticular sites, including the cell surface, participating to receptor activation and remodelling.<sup>32,33</sup>

ERp5 is able to reduce the disulfide bond that binds MICA to tumor cells, thereby releasing MICA and reducing the rate of tumor expansion.<sup>36</sup> In chronic lymphocytic leukemia, ERp5 is translocated to the surface of malignant cells, where it forms a transitory disulfide bond with MICA from which soluble MICA is released upon proteolytic cleavage.<sup>37</sup> ERp5 is also expressed on the surface of myeloma cells, another setting in which it has been associated with MICA shedding.<sup>38</sup> Recently, we have reported that the overexpression of ERp5 in Hodgkin's lymphoma lesions is associated with an impaired NKG2D-mediated immune response.<sup>6</sup> The clinical relevance of ERp5 levels on the surface of tumor cells is highlighted by the association between natural anti-ERp5 antibodies and response to immunotherapy in patients affected by diverse solid tumors and myeloid leukemia.<sup>39</sup> This raises the possibility that ERp5 might serve as a target for therapeutic monoclonal antibodies.

### Induction of NKG2DLs and Blockage of NKG2DL Release to Rescue Stress-Induced Immunity

In several types of lymphoma, the tumor microenvironment seems to be crucial for the survival and proliferation of malignant cells.<sup>1-4</sup> In follicular lymphomas, neoplastic cells reside and accumulate within follicular structures in close association with lymphocytes and dendritic cells, as it occurs in the germinal center of healthy lymph nodes (LNs). In contrast, neoplastic B cells are rarely encountered in interfollicular areas. This suggest that lymphoma cells require a germinal center-like microenvironment to expand.<sup>3</sup> In classical Hodgkin's lymphomas, malignant RS cells account for less than 1% of the tumor tissue. Rather, neoplastic lesions are mainly composed of lymphocytes, eosinophils and macrophages, pointing to an unsuccessful inflammatory response against the tumor. Neoplastic cells isolated from both Hodgkin's and non-Hodgkin's lymphomas can grow in culture much better when seeded onto a layer of stromal cells. Likewise, stromal and nurse-like cells are responsible for the increased survival of

cultured chronic lymphocytic leukemia cells. Nonetheless, cells from all these lymphoproliferative diseases have been described to express stress-induced molecules, including NKG2DLs, on their surface,<sup>13,14,18-20</sup> although this not always leads to effective anticancer immune responses.

### Induction of NKG2DLs

We have shown that  $\gamma\delta$  T lymphocytes belonging to the V $\delta$ 1 subset are expanded in patients with chronic lymphocytic leukemia and non-Hodgkin's lymphoma,<sup>5,15</sup> proliferating in response to NKG2DL expressed by cancer cells. These  $\gamma\delta$  T cells can exert cytotoxic effects against autologous cancer cells and produce antineoplastic as well as pro-differentiation cytokines, such as TNF $\alpha$  and IL-4.<sup>5,6,15,19</sup> Moreover, circulating V $\delta$ 1 T lymphocytes proliferate and produce IFN $\gamma$  in response to autologous cells that express MICA or ULBPs upon exposure to ATRA or VPA. Of note, IL-4 can be found in the serum of non-Hodgkin's lymphoma patients with good prognosis, while the circulating levels of soluble NKG2DLs correlate with disease progression in multiple myeloma, chronic lymphocytic leukemia, non-Hodgkin's lymphoma and acute myeloid leukemia patients.<sup>5,6,15,16,38</sup> In particular, soluble MICA and soluble ULBP2 have been shown to constitute negative prognostic markers in multiple myeloma patients and to allow for the identification of early-stage chronic lymphocytic leukemia patients at risk of disease progression.<sup>15,38,40</sup> We have recently described that, in classical Hodgkin's lymphoma, LN mesenchymal stromal cells (LNMSCs) express very low levels of MICA, MICAB, and ULBPs coupled to high levels of ERp5 and ADAM10,<sup>6</sup> both of which are related to the shedding of soluble NKG2DLs (see above).<sup>36-42</sup> These enzymes were expressed by both LNMSCs and RS cells. Moreover, MICA and ULBP3 could be detected in the culture supernatants of LNMSCs and RS cells. NKG2DL-negative RS cells could not be killed by CD8<sup>+</sup>  $\alpha\beta$  or  $\gamma\delta$  T cells, but tumor cell killing could be partially restored by treating RS cells with VPA, enhancing NKG2DL expression on the cell surface.<sup>6,15,16</sup> Of note, both LN cells and LNMSCs were found to secrete high amounts of transforming growth factor  $\beta$ 1 (TGF $\beta$ 1), which is able to inhibit the expression of NKG2D on the cell surface. Conversely, IL-15, which promotes the expression of NKG2D, was downregulated in the stroma of Hodgkin's lymphoma lesions.<sup>6,43</sup> Thus, at least in the case of Hodgkin's lymphoma, the tumor microenvironment is prone to impede stress-related immunity, either through the shedding of NKG2DLs or through the downregulation of NKG2D receptors.

### Blocking of sheddases

Based on the stress-related immunity hypothesis, it is tempting to speculate that, for avoiding lymphomagenesis, the LN microenvironment must be able to induce a cellular response against stress-related antigens expressed by neoplastic cells, for instance by limiting the release of these antigens as soluble molecules. The amount of stress-related antigens expressed on the surface of cancer cells increases upon malignant transformation. Along similar lines, these molecules are upregulated on the surface of stromal cells of the tumor microenvironment as a consequence of chronic inflammation (Fig. 3).<sup>7-10</sup> Such a physiopathological induction as well as the pharmacological upregulation of

NKG2DLs (for instance, as achieved with ATRA or VPA) is counteracted by the enzymatic activity of different sheddases, including ERp5, ADAM10, and ADAM17, leading to the release of these ligands as soluble fragments and hence to the inhibition of cancer cell recognition by a branch of the immune system (Fig. 3).<sup>14–18,36–42</sup> Of note, a correlation between the levels of soluble NKG2DLs and tumor stage has been reported for chronic lymphocytic leukemia patients.<sup>15,40</sup> Moreover, the progression of solid tumors seems to inversely correlates with the enzymatic activity of sheddases.<sup>39,41,44</sup> The proteolytic cleavage of MICA has been shown to depend on the thiol isomerase ERp5, which binds to MICA  $\alpha 3$  domain, as well as on ADAM10 or ADAM17, which can also cleave ULPBs.<sup>36–43</sup> The overexpression of these sheddases has been reported in patients with multiple myeloma and other tumors, including Hodgkin's and non-Hodgkin's lymphoma.<sup>6,15,38</sup> Blocking ERp5 would be possible with antagonistic peptides that mimic the  $\alpha 3$  domain of MICA and hence impede the interaction of the enzyme with its substrate.<sup>44–46</sup> Conversely, pharmacological inhibitors of ADAM10 and ADAM17 with some degree of selectivity have already been developed.<sup>47–49</sup>

### Concluding Remarks

Based on these considerations presented in this review, redirecting stress-elicited immune responses and avoid escape strategies set in place by malignant cells might constitute a novel, valuable therapeutic paradigm against lymphoma that mainly

operates on the tumor microenvironment. In summary, the rationale would be to induce the exposure of stress molecules on the surface of malignant cells, to inhibit the enzymatic activity of sheddases, and to modulate mesenchymal stromal cells to shift cytokine secretion toward a  $T_H1$ , rather than immunosuppressive, profile (Fig. 3).<sup>50–53</sup> First, the expression of membrane-bound NKG2DLs by cancer cells might be stimulated by drugs such as ATRA and VPA or, as recently reported, by proteasome inhibitors,<sup>5,6,15,54–56</sup> all of which have already been approved for the treatment of hematological malignancies. Second, ERp5, ADAM10 and ADAM17 may represent targets for selective inhibitors of their enzymatic activity.<sup>44–49</sup> Recent preclinical data based on the use of ADAM17 inhibitors for the treatment of solid neoplasms appear to support this notion.<sup>57</sup> Third, potentiating NKG2D-mediated antitumor immune responses by the RNA interference (RNAi)-mediated depletion of TGF $\beta$ 1 has been reported to results in the inhibition of tumorigenicity in vivo.<sup>58</sup> In addition, N-BPs may promote the secretion of  $T_H1$  cytokines in the tumor microenvironment,<sup>59–63</sup> hence stimulating NKG2D expression and boosting the function on effector lymphocytes.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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