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REVIEW

Granulysin and its clinical significance as a biomarker of immune response and NK cell related neoplasms

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Abstract

Granulysin is a cytotoxic granular protein that was identified from human T cells by using the gene subtraction method in 1987. Based on its amino acid homology, granulysin belongs to the saposin-like protein family. The bioactive 9-kDa form of granulysin is processed from the 15-kDa pro-product in the cytoplasmic granules. It is expressed in CD8-positive αβT cells 5 d after mitogenic stimulation and constitutively in natural killer (NK) cells and γδT cells, although regulation of its expression has not yet been precisely determined. The 9-kDa granulysin form has anti-microbial activity against microorganisms such as bacteria, fungi, mycobacteria and parasites, as well as tumoricidal activity against some tumors at 1-10 μmol/L concentrations. Granulysin is secreted in both Ca-dependent and -independent manners. In sera, only the 15-kDa form is detectable and is expected to be a biomarker for immune potency, acute viral infection, anti-tumor immune reaction, acute graft *vs* host disease, and NK cell associated neoplasm.

Key words: Granulysin; Saposin-like protein family; Natural killer cell; Cytotoxic T lymphocyte

Core tip: Granulysin is a cytotoxic granular protein expressed in cytotoxic T cells, natural killer (NK) cells and γδT cells, and has anti-microbial activity against microorganisms such as bacteria, fungi, mycobacteria and parasites, as well as tumoricidal activity against some tumors. It is secreted constitutively and in a trigger-dependent manner. Clinically, serum granulysin is a unique biomarker for immune response, immune capacity and NK cell related neoplasms.

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INTRODUCTION

Granulysin is a cytotoxic granular protein that was identified in human T cells by using the gene subtraction method in 1987[1]. While granulysin is not expressed in resting αβT cells, it is constitutively expressed in natural killer (NK) cells and γδT cells. In contrast to other cytotoxic granular proteins, such as perforin and granzyme, granulysin is expressed in αβT cells later following antigenic stimulation (Figure 1). In this review, we summarize the structure, the *in vivo* and *in vitro* functions, and the regulation of expression of granulysin. Furthermore, we

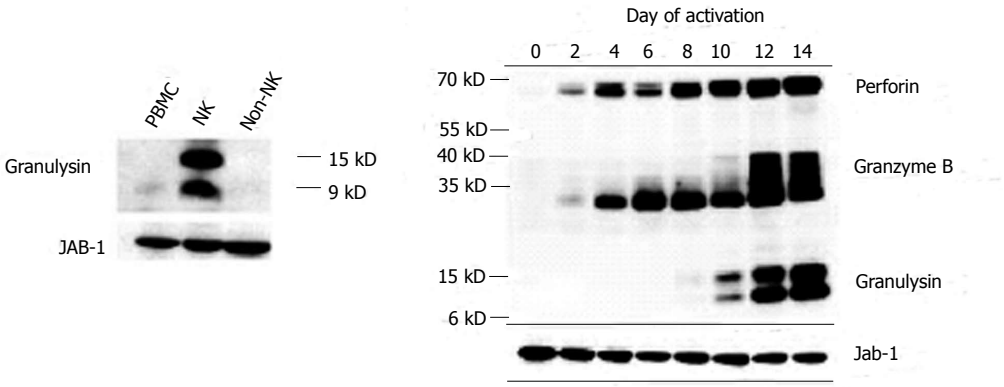


Figure 1 Expression of granulysin after T cell activation analyzed by western blotting. Granulysin is expressed later compared to perforin and granzyme B after T cell activation. In natural killer (NK) cells, granulysin is constitutively expressed. Jab-1 (c-Jun activation domain-binding protein-1) is used as internal control. PBMC: Peripheral blood mononuclear cell.

Table 1 Saposin-like protein family members and their proposed functions

Family member	Function	Identity to 9-kDa granulysin (amino acid)[1]	Similarity to 9-kDa granulysin (amino acid)[2]
Saposin A	Sphingolipid hydrolase activator	21	46
Saposin B	Sphingolipid hydrolase activator	19	50
Saposin C	Sphingolipid hydrolase activator	19	53
Saposin D	Sphingolipid hydrolase activator	20	46
Pulmonary surfactant protein B	Lipid organization in pulmonary surfactant	19	53
Acyloxyacyl hydrolase	Phagocytic cell lipase	22	50
Acyloxyacyl hydrolase	Sphingolipid hydrolase	13	42
Amoebapore A	Pore-forming Entamoeba histolytica granule protein	16	47
Amoebapore B	Pore-forming Entamoeba histolytica granule protein	13	42
Amoebapore C	Pore-forming Entamoeba histolytica granule protein	18	47
NK-lysin	Lytic porcine T cell and NK cell granule protein	35	66
Granulysin	Lytic human T cell and NK cell granule protein	100	100

[1]Identity denotes the percentage of identical amino acids; [2]Similarity denotes amino acids that share chemical properties, for example, charge or hydrophobicity. NK: Natural killer.

present results examining granulysin as a biomarker and discuss future investigations with granulysin.

STRUCTURE AND FUNCTION

Two isoforms of granulysin with molecular weights of 15-kDa and 9-kDa, respectively, have been identified and the biologically active 9-kDa isoform is derived from the 15-kDa isoform by intracellular processing (Figure 1). Based on amino acid sequence homology, the 9-kDa granulysin protein belongs to the saposin-like protein (SAPLIP) family containing the sphingolipid hydrolase activators of the central nervous system (Table 1)[2,3]. The gene is located at chromosome 2p11.2 in humans and homologues have been identified in pig, horse and cow. The absence of a homologous gene in rodents (mice) makes it difficult to investigate its physiological significance using rodent models[4,5]. Recently, Huang *et al*[6] and Liu *et al*[7] characterized a mouse model in which the human granulysin gene was introduced. This chimeric mouse model may be useful for the advanced functional analysis of granulysin in the future. Granulysin has cytotoxic activity similar to other SAPLIP family proteins such as amoebapore A, B, C (*Entamoeba histolytica* pore-forming protein) and NK-lysin (a porcine lytic granule protein)[8].

Crystal structure analysis of granulysin suggests that it consists of five α-helices (Figure 2). Although a physiological cell surface receptor for granulysin has not yet been identified, it is speculated that granulysin folds into a structure in which the positively charged active site interacts with negatively charged sites on bacterial or tumor cells and exhibits its cytotoxic activity. It is hypothesized that granulysin molecules aggregate on the target cell surface in an electric charge energy-dependent manner, and they rotate in the direction from α-helix1 to α-helix2 to α-helix3, pierce the cell membrane, and enter the cell[9,10]. Whereas synthetic peptides consisting of α-helix2 and α-helix3 kill bacterial and tumor cells, peptides consisting of α-helix3 alone kill bacterial, but not tumor cells. Substitution of cysteine residues in α-helix2 and α-helix3 with serine residues deprives the synthetic peptides of cytotoxic activity for human tumor cells, and replacing arginine residues with glutamine residues also abolishes its activity. When the cysteine residue is in the non-reduced state, the cytotoxic activity for tumor cells is lost while the cytotoxic activity for bacteria remains unaffected[10,11] (Figure 2B), suggesting that the reduced cysteine residue is essential for the cytotoxic activity for tumor cells. Substitution of D-amino acids 32-42 with L-amino acids maintains the same cytotoxic activity but induces resis-

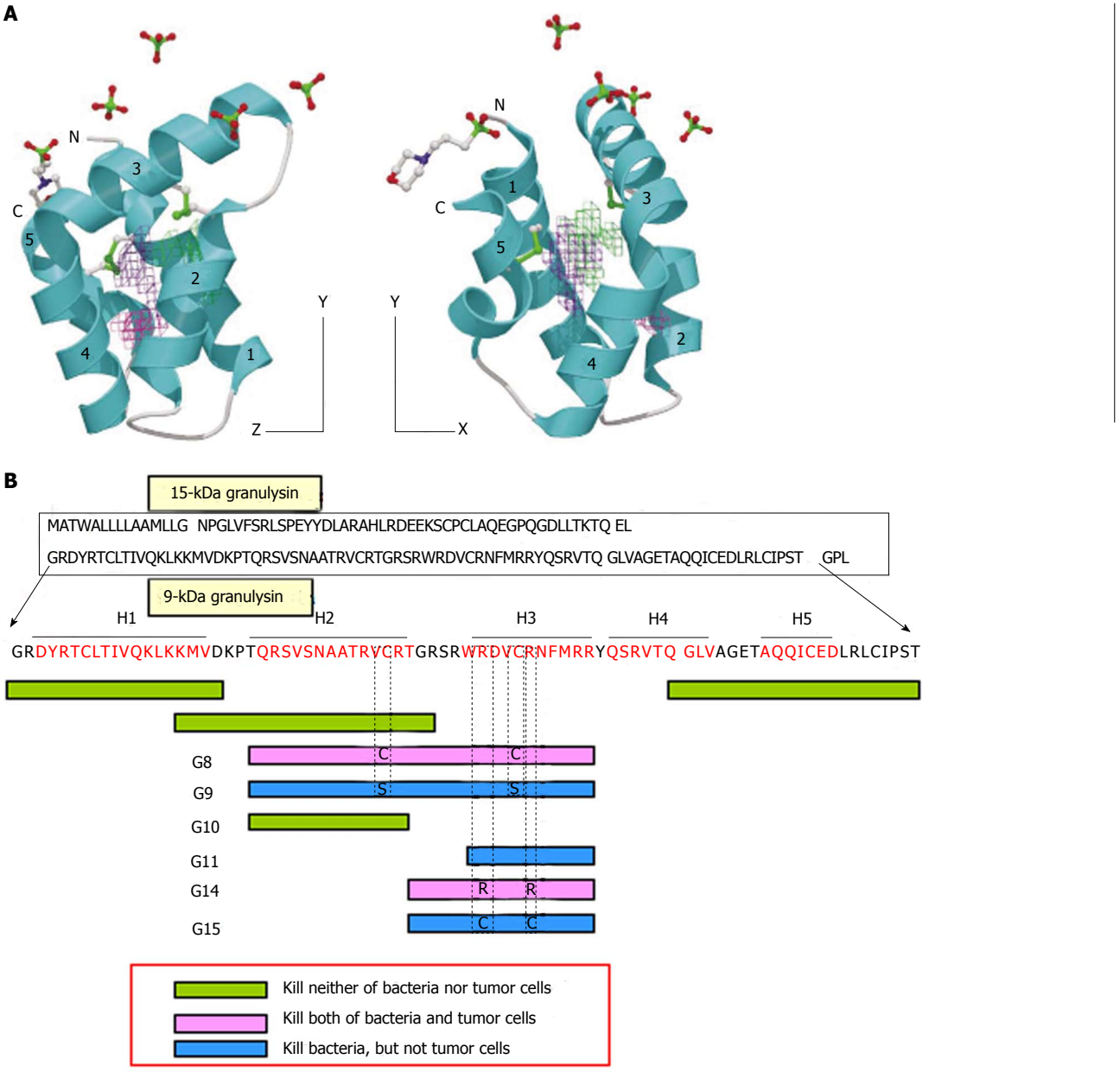


Figure 2 Granulysin. A: 3-D structure model of 9-kDa granulysin. Granulysin consists of five a-helices. Cytotoxic active site ranges between helix-2 and helix-3, in which positive electric charges are located[9]; B: Scheme of cytotoxic active site in granulysin. Amino acid sequence of granulysin and its biologically active site are illustrated. See STRUCTURE AND FUNCTION in the text for detailed explanation.

tance to inactivation by trypsin and the serum. These observations raise the possibility for the development of new synthetic peptides with cytotoxic activity, specifically for bacteria or for the development of biologically active peptides that can act for a long time *in vivo*[13].

EXPRESSION AND CYTOTOXIC ACTIVITY

Granulysin is expressed by activated cytotoxic T lymphocytes (CTL), mainly by CD8-positive T lymphocytes and some CD4-positive T lymphocytes[1,14]; it is also expressed by NK cells and γδT cells constitutively[15,16]. B cells and granular leukocytes do not express granulysin, but monocytes may express granulysin when activated. There is also a report indicating that granulysin was expressed in a megakaryocyte cell line, but whether it is expressed in platelets remains unclear[17].

Granulysin is synthesized as 15-kDa protein in the cytoplasm. The N-terminal amino acid sequence is thought to contain a transportation signal that directs granulysin to a cell granule. Some of the amino acids at the N- and C-termini are removed by unknown mechanisms within the cell granule to produce the active 9-kDa protein[14]. When the pH within the cytosomal granules is increased due to the presence of the H^+-ATPase inhibitor concanamycin A, processing to the 9-kDa protein is inhibited. Furthermore, against artificial cell membranes, the membrane injury activity of the 9-kDa granulysin is markedly reduced at pH 6.4 or lower. This most likely

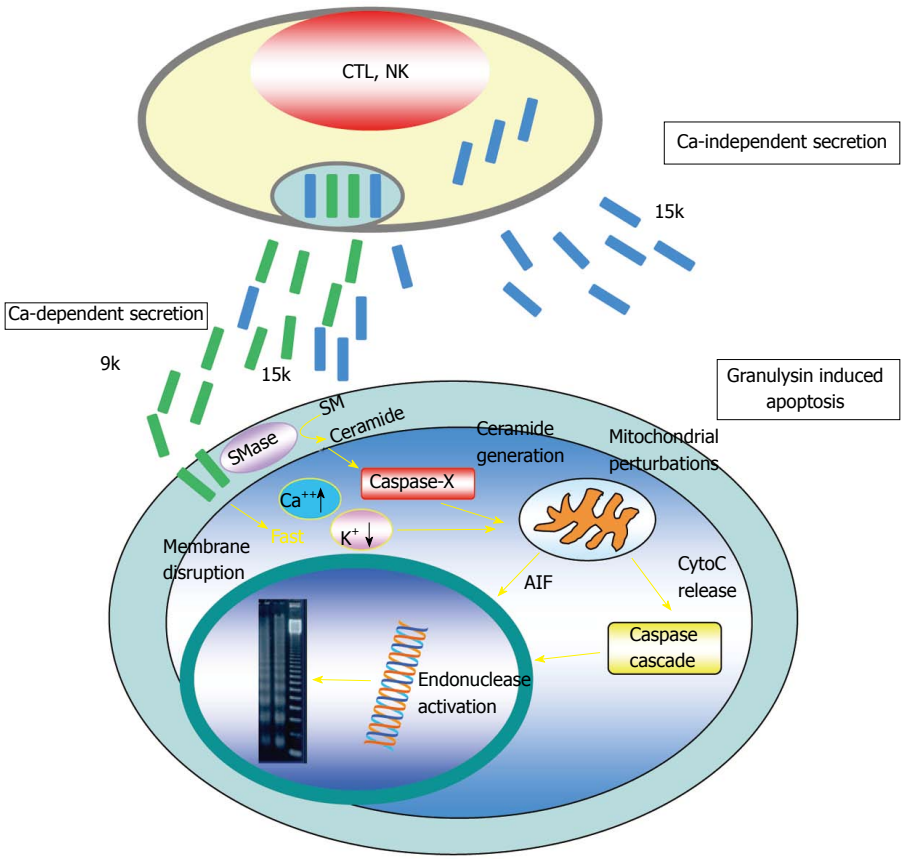


Figure 3 Schematic model of how granulysin kills target cell. (Cited from ref.[48] revised by author). NK: Natural killer; AIF: Apoptosis-inducing factor; CTL: Cytotoxic T lymphocytes.

explains why active 9-kDa granulysin does not cause autolysis in cytotoxic granules[18-20]. The CTL and NK cell granules have similar amounts of both the 15-kDa and the 9-kDa molecules, but while the 9-kDa molecules stay within the cytotoxic granules, the 15-kDa molecules are secreted constantly (Figure 3). Most of the 15-kDa molecules are thought to be secreted *via* an alternative pathway without entering the cytotoxic granules. Since the 15-kDa molecule does not have cytotoxic activity, its physiological role is currently not understood. Recently, it has been reported that 15-kDa granulysin induces differentiation of monocytes to dendritic cells and may modulate the immune response[21]. However, the 15-kDa molecule is detectable in serum and its potential significance as a biomarker has been recently reported.

9-kDa granulysin is released when co-cultured with target K562 cell and its release is prohibited by depletion of calcium, indicating Ca-dependent and trigger-dependent excretion of 9-kDa granulysin (see GRANULYSIN AS A BIOMARKER).

The 9-kDa molecule can kill gram-negative and gram-positive bacteria, fungi, parasitic worms, acid-fast bacilli and malarial parasites directly, but not intracellular parasites in the absence of perforin. Some studies also suggest that granulysin cannot enter the cytoplasm of the parasite in the absence of perforin[22].

Hata *et al*[23] reported that granulysin inhibits the growth of the varicella virus and induces apoptosis in infected cells. Granulysin-expressing CD4-positive T lymphocytes also kill *Cryptococcus neoformans*. Recently, Ochoa *et al*[24] reported that CD4-positive T lymphocytes infiltrating the lesions in leprosy patients express granulysin and are associated with control of the leprosy bacillus. Granulysin also has been reported to possess cytotoxic activity against some tumor cells[25]. The cytotoxic effects of granulysin against Jurkat cells are mediated by the entry of extracellular calcium into the cell after cell membrane destruction by granulysin, thereby inducing the release of intracellular calcium. Intracellular potassium (K) is reduced by a calcium-dependent K pump. This results in injury to the mitochondria and inhibits oxidative phosphorylation. With the release of cytochrome c and apoptosis-inducing factor (AIF) from the mitochondria, caspases are activated within several minutes and apoptosis is induced. This model of apoptosis induction by granulysin is evidenced by the fact that inhibition of the calcium-dependent K pump *via* suppression of intracellular calcium release inhibits apoptosis induction. In addition, granulysin also induces late caspase activation through an alternative pathway by activating membrane sphingomyelinase and inducing ceramide formation[9,10,26,27] (Figure 3).

The 9-kDa granulysin also has pro-inflammatory functions similar to defensins and acts as a chemotactic factor for CD-4 positive and CD8-positive T lymphocytes and monocytes. This chemotactic activity is affected at 10 nM concentrations of granulysin, which is much lower than that required for its cytotoxic activity (1-10 μmol/L). It is speculated that granulysin acts through a

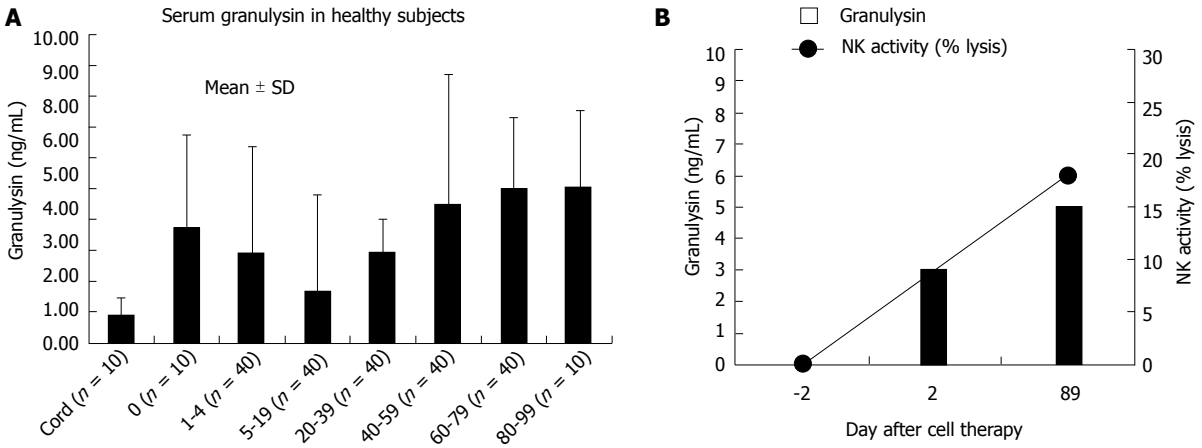


Figure 4 Serum granulysin in healthy subjects (A, see GRANULYSIN AS A BIOMARKER in the text) and relationship with natural killer cell activity (B). Serum granulysin is increased along with the recovery of natural killer (NK) cell activity in infant with combined immunodeficiency after cell therapy.

G protein conjugate receptor because the chemotaxis can be inhibited with pertussis toxin, but the details of the receptor are as yet unknown. The 9-kDa granulysin acts on monocytes and a cell line with monocytic-lineage (U937), and induces RANTES, monocyte chemotactic protein (MCP) 1, MCP-3, Macrophage inflammatory protein-1α, Interleukin (IL)-10, IL-1, IL-6 and interferon (IFN)-α[28].

REGULATION OF GRANULYSIN EXPRESSION

In comparison to its physiological functions, the regulation of granulysin expression remains to be elucidated.

The binding sites for activator protein-1 (AP-1), CCAAT/enhancer binding protein β (C/EBPβ) and nuclear factor kappa B (NF-κB) have been identified in the promoter region of the granulysin gene. Using the reporter assay system in which the monocyte-lineage cell line THP-1 is stimulated with *Acholeplasma laidlawii* (*A. laidlawii*) (mycoplasma), Kida *et al*[29,30] reported that two AP-1 binding sites (from -277 to -271 bp and from -96 to -86 bp) and the C/EBPβ binding site (from -1003 to -990 bp) are important for regulation of transcription, and that the former acts positively while the latter acts negatively. In the system described above, although *A. laidlawii* stimulation activated NF-κB through toll-like receptor 2 (TLR2) and the p50 homodimer bound to the NF-κB region, there was no influence on granulysin transcription[30].

NK cells express granulysin and IL-2 receptor β and γ chain constitutively. The expression of granulysin mRNA and protein was not altered after stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin, IL-2 or IFN-α[31]. Expression of granulysin was increased in CD8-positive T lymphocytes five days after antigen stimulation as mentioned above. Endsley *et al*[32] reported that CD4-positive T lymphocytes did not express granulysin even after PMA and ionomycin stimulation, whereas Zheng *et al*[33] reported that CD4-positive T lymphocytes expressed granulysin in the presence of IL-2 through PI3K and STAT5 activation, although anti-CD3 stimulation alone did not induce granulysin expression[33]. Transient activation of STAT5 occurred 30 to 60 min after IL-2 stimulation, following which a reactivation of STAT5 was observed after 3 d that induced IL-2 receptor β expression. Consequent interaction of IL-2 with IL-2 receptor β activated PI3K and induced granulysin[34]. Granulysin expression is inhibited by the anti-IL-2 receptor β antibody but not by the anti-IL-2 receptor α antibody, indicating the importance of IL-2 receptor α in inducing granulysin expression. Evidence for STAT5-controlled expression of granulysin also comes from the observation that patients with HIV infection have an increased susceptibility to *Cryptococcus neoformans*, which is probably due to insufficient activation of STAT5 and PI3K in CD4-positive T lymphocytes, resulting in reduced expression of granulysin[33].

Scherer *et al*[35] examined the expression of granulysin mRNA after stimulation with tuberculin purified protein derivative (PPD) in lymphocytes from bovine immunized with Bacille de Calmette et Guerin (BCG)[35]. Compared to non-immunized bovine controls, granulysin mRNA was increased more than 50 times in CD8-positive T lymphocytes 12 h after immunization and 48 h after immunization in CD4-positive T lymphocytes. Furthermore, whereas the mRNAs of perforin, IFN-γ and Fas-ligand in CD4-positive T lymphocytes increased after PMA + ionomycin stimulation, as well as after PPD stimulation, granulysin mRNA was not enhanced after PMA + ionomycin stimulation, corroborating the previous observation by Endsley *et al*[32].

GRANULYSIN AS A BIOMARKER

As mentioned above, the 15-kDa and 9-kDa granulysin forms exist at approximately a 1:1 ratio in cells. The precise mechanism of this conversion and its regulation is unknown. The non-active 15-kDa precursor of granulysin is secreted constantly, but the active 9-kDa form is released in a calcium-dependent manner. Based on the observation that the 9-kDa form is not detected in the culture medium even after *in vitro* stimulation, it

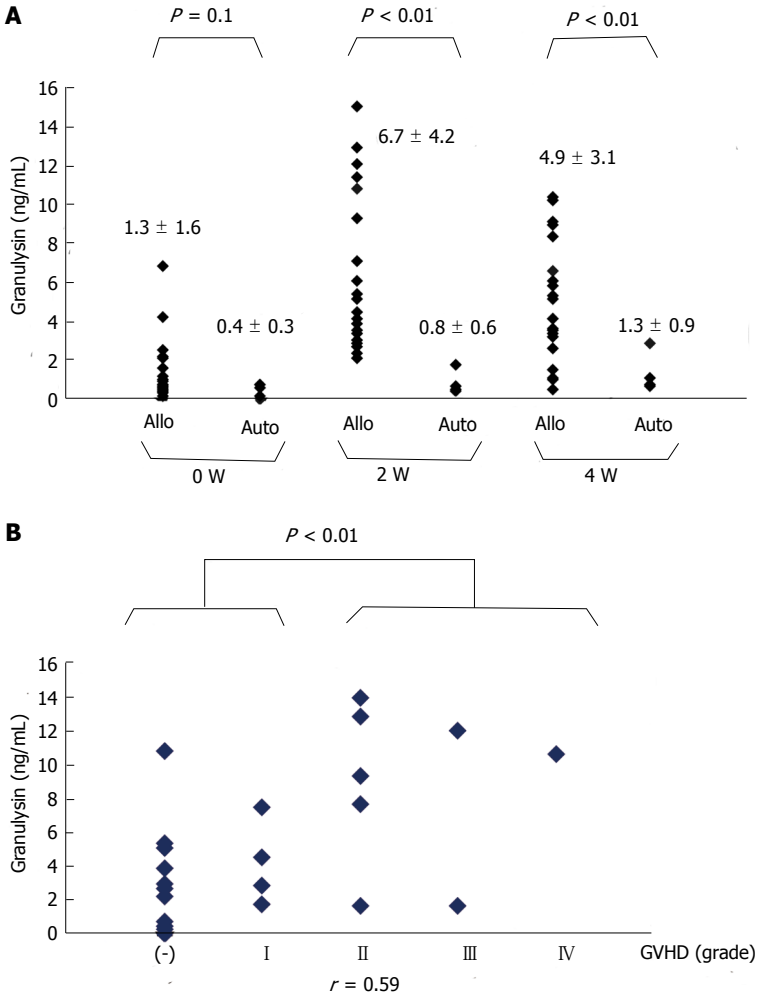


Figure 5 Trend of serum granulysin. A: In patients with allo-hematopoietic stem cell transplantation (HSCT) (n = 21) and auto-HSCT (n = 5). Serum granulysin is elevated 2 wk after allogeneic hematopoietic stem cell transplantation, but not autologous hematopoietic stem cell transplantation; B: With the grade of graft-versus-host disease (GVHD). Serum granulysin and the grade of GVHD were plotted and their correlation coefficient was calculated (r = 0.59). The serum granulysin level of patients with grade 2 or more is significantly higher than that of patients with grade 1 or no GVHD.

is possible that the active form is immediately adsorbed, consumed or destroyed. By contrast, the 15-kDa form is easily detected in the culture medium and serum and is increased after *in vitro* stimulation[36]. This indicates that the 9-kDa and 15-kDa forms are released together after stimulation, but only the 15-kDa form is detected. Any increase in the release of the 9-kDa form is therefore estimated to arise indirectly from the increased amount of the 15-kDa form, since inhibition of cellular secretion using Brefeldin A increased the intracellular levels of granulysin in CTL and NK cells but did not affect intracellular perforin and granzyme levels[36].

Granulysin as a biomarker in cell-mediated immunity

To estimate the levels of serum granulysin in healthy subjects, a novel, highly sensitive Enzyme Linked Immuno-Sorbent Assay method was used (Figure 4A). Levels of granulysin gradually increase with aging and are extremely low in umbilical cord blood. These levels reflect the levels of constitutively secreted granulysin and can be correlated either with NK cell activity or the number of NK cells and γδT cells, which constitutively express granulysin[36]. It is well known that NK activity increases with ageing until the age of 40 and decreases thereafter. The discrepancy between granulysin level and NK activity after the age of 40-50 is not well explained. One possibility is that the ratio of conversion from 15-kDa to 9-kDa changes after the age of 40. We have no data concerning this issue, which remains to be investigated.

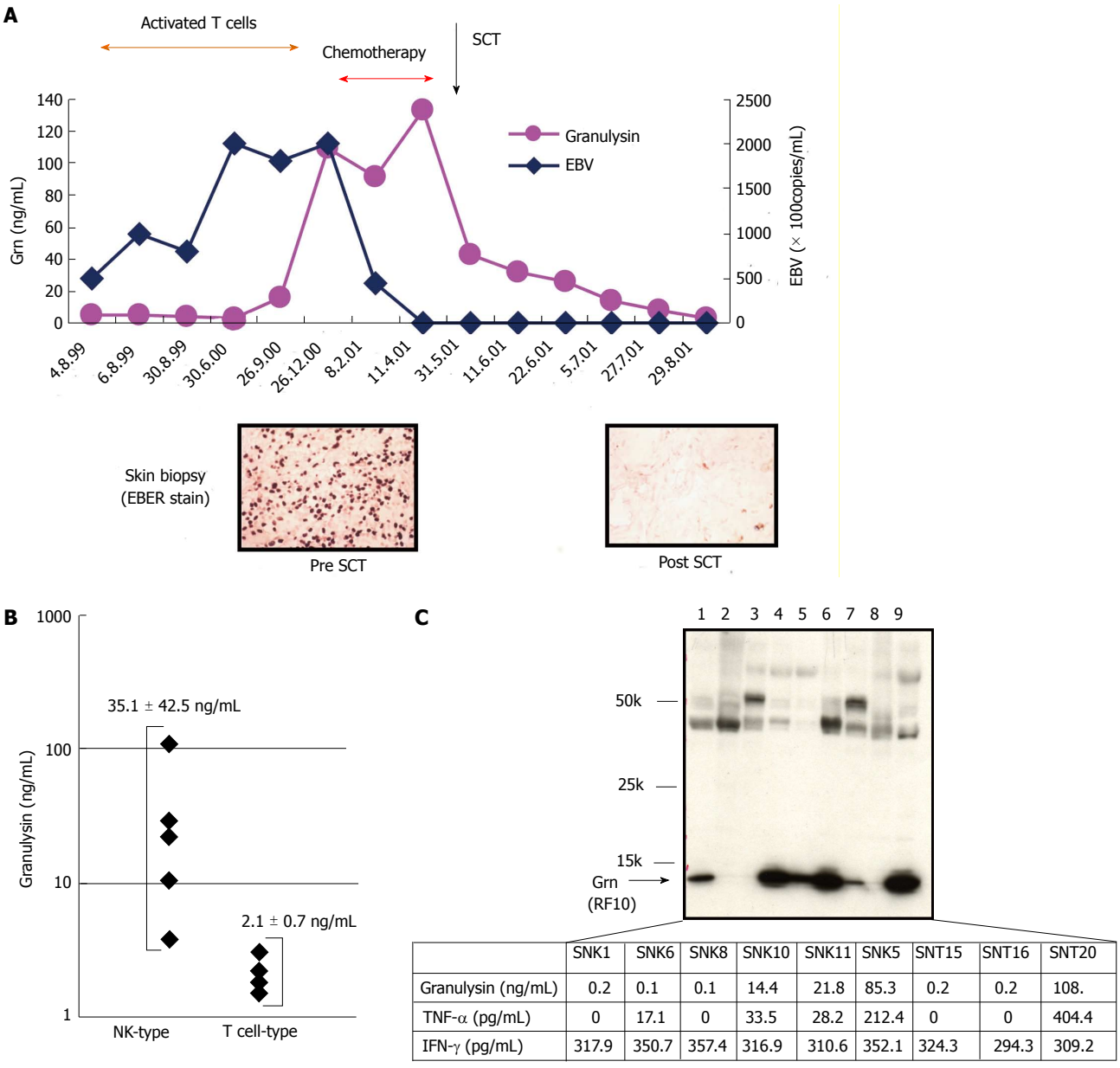
In infants with severe immunodeficiency without NK cells, serum granulysin was undetectable and became measurable when a cell-mediated immunity function was restored by hematopoietic stem cell transplantation (unpublished data). After transfusion of autologous *in vitro*-activated T cells back into a patient with incompetent cell-mediated immunity, levels of serum granulysin were increased along with the recovery of NK activity (Figure 4B)[36]. These observations indicate that serum granulysin is useful as a new biomarker for evaluation of cell-mediated immunity.

Granulysin as a biomarker in acute virus infection

Infectious mononucleosis is an acute disease resulting from primary Epstein-Barr (EB) virus infection, in which activated CD8-positive CTLs are increased in the peripheral blood. Increased CD8-positive CTLs are reactive and cytotoxic against EBV-infected B lymphocytes. Serum granulysin is markedly increased during an acute phase of infectious mononucleosis and becomes normalized in convalescence[36].

Granulysin as a biomarker of hemophagocytic lymphohistiocytosis

Hemophagocytic lymphohistiocytosis is a histiocytosis-



	SNK1	SNK6	SNK8	SNK10	SNK11	SNK5	SNT15	SNT16	SNT20
Granulysin (ng/mL)	0.2	0.1	0.1	14.4	21.8	85.3	0.2	0.2	108.
TNF-α (pg/mL)	0	17.1	0	33.5	28.2	212.4	0	0	404.4
IFN-γ (pg/mL)	317.9	350.7	357.4	316.9	310.6	352.1	324.3	294.3	309.2

Figure 6 Serum granulysin. A: Clinical course and trend of serum granulysin in a natural killer (NK) cell type chronic active EB virus infection (CAEBV) patient. For detailed explanation, see GRANULYSIN AS A BIOMARKER, 6: Granulysin in NK cell-related tumors or neoplasm in the text; B: Serum granulysin in patients with NK type and T cell type CAEBV, serum granulysin in patients with NK type (n = 5) and T cell type (n = 4) chronic active EB virus infection. Only in NK type CAEBV, serum granulysin is significantly elevated. Serum granulysin in patients with NK type (n = 5) and T cell type (n = 4) chronic active EB virus infection. Only in NK type CAEBV, serum granulysin is significantly elevated. C: Expression of granulysin and cytokine production in EB virus infected cell lines (SNK1,5,6,10,11: NK cell type, SNT8,15,16,20: γδT cell type). Western blotting was performed by using a monoclonal antibody, RF10 which reacts with 15-kDa but not 9-kDa granulysin. TNF-a and IFN-g in the culture supernatant were assayed by ELISA method. TNE: Tumor necrosis factor; INF: Interferon.

related disease characterized clinically by fever, pancytopenia, hepatosplenomegaly and hyperlipidemia. T cells are strongly activated during the acute phase of hemophagocytic lymphohistiocytosis (HLH) and levels of Th1 cytokines, such as IL-12, IL-18 and IFN-γ, are abnormally high, which leads to the abnormal activation of macrophages. Serum ferritin and soluble IL-2 receptor (sIL2R) have been reported as clinical markers of HLH. The treatment of HLH includes immunosuppressive therapy, anti-cancer drug chemotherapy and hematopoietic stem cell transplantation in severe cases. We measured serum granulysin in 24 HLH patients prior to treatment and reported that levels of granulysin were extremely high during the acute phase of HLH. Since serum granulysin levels decreased in parallel with disease regression following therapy, granulysin seems to be useful as a novel biomarker of HLH[37].

Granulysin as a biomarker of tumor immunity

Kishi *et al*[38] examined intracellular levels of granulysin and perforin in NK cells of cancer-bearing patients and healthy subjects by flow cytometry. Levels of intracellular granulysin were significantly decreased in cancer-bearing patients, while those of intracellular perforin were not

changed compared to healthy subjects[38]. Spontaneous regression of neuroblastoma has been observed frequently in infants younger than one year old. We previously reported a case study of an infant with neuroblastoma IVS who showed dramatic spontaneous regression. During the regression, serum granulysin and IFN-γ levels were transiently and markedly elevated[39]. Although interpretation of these observations is difficult, it seems that serum granulysin is related to tumor immunity and could be a novel biomarker of tumor immunity.

Granulysin as a biomarker in acute graft-versus-host disease

Elevated granulysin mRNA levels have been reported in infiltrating cells of acutely rejected kidneys from renal transplant patients[40]. To examine whether serum granulysin is a marker of acute graft-*vs*-host disease (GVHD) in hematopoietic stem cell transplantation (HSCT), we first isolated alloantigen-specific CTLs and confirmed that serum granulysin was released in an allospecific manner *in vitro*. Next, we examined serum granulysin in autologous and allogeneic hematopoietic stem cell transplantation cases. Serum granulysin was significantly and transiently increased in allogeneic HSCT 2 wk after SCT (6.7 ± 4.2 ng/mL), but not in autologous HSCT (0.8 ± 0.6 ng/mL) (Figure 5A). We also examined and found a significant correlation in the severity of acute GVHD and levels of granulysin (Figure 5B). Efficacy of soluble IL-2 receptor (sIL2R) has been reported as a biomarker of acute GVHD[41]. However, there were cases in which the change of sIL-2R levels and the symptoms of GVHD did not correlate in clinical settings. As per our observations, sIL-2R correlated well with serum granulysin during the first two months after HSCT, but serum granulysin reflected GVHD symptoms much better than sIL-2R thereafter. This discrepancy seems interesting in understanding the complicated pathology of GVHD and highlights the utility of serum granulysin as a biomarker that is distinct from sIL-2R for acute GVHD.

Granulysin in NK cell-related tumors or neoplasms

αβT cells express granulysin only after being activated and/or on maturation to CTLs. However, as mentioned above, granulysin is expressed constitutively in NK cells and γδT cells. Based on the foregoing observations, we examined the possibility of evaluating granulysin as a marker for NK-related tumors. Chronic active EB virus infection (CAEBV) is a disease with poor prognosis, presenting with fever, mosquito hypersensitivity, lymphadenopathy and hepatosplenomegaly, in which T cells or NK cells infected with EB virus are detected in the peripheral blood, and is usually classified as either the NK cell type or the T cell type. Interestingly, CD4-positive αβT cells are infected with the T cell type of EB virus. NK cell type CAEBV has been named hydroa vacciniforme because it is characterized clinically by varicelliform eruptions characterized histologically by infiltrating EB virus-positive cells. CAEBV frequently progresses to hemophagocytic syndrome or malignant lymphoma after a chronic clinical course. Figure 6A shows the levels of serum granulysin and blood EB viral genome in a patient with NK cell type CAEBV during a long-term clinical course. Serum granulysin and blood EB viral genome increased with progress of the disease. While blood EB viral genome decreased in response to chemotherapy, serum granulysin levels normalized only after allogeneic hematopoietic stem cell transplantation. A comparison of serum granulysin levels in NK cell type and T cell type CAEBV patients indicated that serum granulysin was significantly increased only in NK cell type patients (Figure 6B). Expression of granulysin was also confirmed by analyzing NK cell and γδT cell lines established from CAEBV patients[42]. CD4-positive αβT cell lines have not yet been established, but examination of a tumor tissue from a patient who presented with an EB virus-positive, CD4-positive lymphoma over the course of CAEBV[43], did not reveal any expression of granulysin (unpublished observations). Interestingly, cell lines with granulysin expression also showed enhanced TNF-α production, although the levels of INF-γ production were the same (Figure 6C). Culturing in the presence of the NF-κB inhibitor did not affect the expression of granulysin in these cell lines (unpublished observation). Sekiguchi *et al*[44] reported that serum granulysin was significantly increased in patients with aggressive NK cell leukemia[44]. Granulysin has also been implicated in the cell death of keratinocytes in Stevens-Johnson syndrome and toxic epidermal necrolysis[45]. Iwai *et al*[46] reported that histological examination of granulysin expression is useful for distinguishing Stevens-Johnson syndrome/toxic epidermal necrolysis from erythema multiforme major.

FUTURE DIRECTIONS

CTL and NK cells secrete the 15-kDa precursor of granulysin constitutively, whereas they secrete both the 15-kDa precursor and the active 9-kDa granulysin forms when exerting cytotoxic activity. Only the 15-kDa form can be detected in sera or culture media, because the active 9-kDa form may be adsorbed, consumed or destroyed rapidly. This characteristic is quite different from that of other cytotoxic granular proteins such as perforin and granzyme, and makes granulysin a unique biomarker of cell-mediated immunity, tumor immunity, infection and GVHD. Structural analysis of granulysin provides the potential for the development of new innovative agents by designing novel analogous proteins using biomolecular technology. The effectiveness of a granulysin-DNA vaccine for tuberculosis in mice models has been recently reported[47]. While many unknowns remain concerning granulysin regulation and function, the combination of novel biotechnological methods will make it possible to develop novel immune, anti-cancer and anti-infection treatment strategies. One difficulty for granulysin research comes from the fact that there is no homologous gene for granulysin in mice. Although granulysin was discovered in 1987, a new report that granulysin is associated with the onset of Stevens-Johnson syndrome

has refreshed interest in granulysin research. The clinical analysis of granulysin as a biomarker has only just begun and it is expected that new findings will be obtained in the future through both basic and clinical studies.

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