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Abstract

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Keywords	Granulysin; Chemotaxis; Cytotoxicity
Taxonomy	Gamma Delta T-Cell, Immune System, Innate Immunity, Migration
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1. Structure and subcellular location of granulysin

Granulysin was first discovered in humans by Jongsta *et al.* whilst looking for genes which occurred late after T cell activation[1]. Using the technique of subtractive hybridisation, granulysin was initially identified within $CD4^+$ and $CD8^+$ αβ T cells, where expression was observed to occur 3-5 days following activation. Several years later, granulysin was also identified within populations of NK cells, where expression was found to be constitutive[2]. Recently, further research has shown that while granulysin is expressed in virtually all NK cell subsets, it is found in a higher percentage of $CD8^+$ T cells as compared to $CD4^+$ T cells, and is also mainly confined to the effector memory subset of these cytotoxic T cells[3]. Granulysin has also been detected in a wide range of additional cytotoxic immune cells, including NKT cells, CD1-restricted cells and $V\delta2^+$ γδ T cells[4–6]. Interesting, granulysin has recently also been identified in type 1 regulatory T cells, which have been shown to have a cytotoxic function, allowing them to eliminate both effector T cells and myeloid cells in a granzyme and granulysin dependent manner[7].

Granulysin has been identified as a member of the saposin-like family of proteins, due to its conserved 3D structure and sequence homology[2]. In particular, it has been found to share considerable homology with the porcine molecule NK-lysin[8]. Linde *et al.* showed that the basic residues arginine and lysine were conserved between NK-lysin and granulysin, and found that these residues were crucial for the antimicrobial activity of NK-lysin, suggesting a similar function for human granulysin[9]. While homologues of granulysin exist in a wide range of other species, it is important to note that neither granulysin or a functional homologue is present in mice, which makes examining the *in vivo* function of this molecule challenging[10].

Human granulysin exists as a 15 kDa molecule, which is subsequently cleaved at both its amino and carboxyl termini to form a 9 kDa protein[8]. The amino terminus of granulysin is thought to be required for accurate intracellular trafficking of this molecule, and cleavage of 15 kDa granulysin into its cytotoxic 9 kDa isoform only occurs once trafficking to an acidic cytotoxic granule is complete[8]. This strictly regulated trafficking and cleavage exists to protect the immune cell from autolysis, and the acidic nature of the cytotoxic granule provides an additional level of protection to the immune cell, as 9 kDa granulysin has been shown only to be active at neutral pH[11].

Initially, the 15 kDa isoform of granulysin was thought to be merely an inactive precursor of the cytotoxic 9 kDa isoform of this molecule. This was perhaps partially due to the

difficulties faced in producing large quantities of recombinant 15 kDa granulysin for use in research. However, recent evidence has suggested that 15 kDa granulysin may have a larger role in the immune system that first thought. The differences in the location and function of the two isoforms of granulysin are summarised in table 1.

One significant finding that adds weight to this hypothesis is the fact that 15 kDa granulysin and 9 kDa granulysin exist in different granules within an immune cell, and require the activation of different pathways in order to be released from the cell. Initial research by Clayberger *et al.* showed through confocal imaging of NK cell populations that 9 kDa granulysin was co-localised with lysosomal marker CD107a, and the cytotoxic molecules granzyme B and perforin, and that on activation of NK cell populations, these cytotoxic granules were released through a granule exocytosis-mediated pathway[12]. Conversely, 15 kDa granulysin was shown to exist within a granule of its own, not governed by directed exocytosis[12]. These findings were later confirmed by Lettau *et al.,* who separated granules containing 15 kDa granulysin and 9 kDa granulysin by density gradient centrifugation[13]. Recently, this group further refined their findings, showing that release of 15 kDa granulysin requires PKC only, while release of 9 kDa granulysin, in addition to other cytotoxic molecules, is dependent on calcium[14]. This suggests that an immune cell can 'sense' which granule to mobilise and release, dependent on the function the cell wishes to elicit. The fact that 15 kDa and 9 kDa granulysin are located in different granules within a cytotoxic cell, and are secreted from the cell through different pathways suggests that 15 kDa granulysin may have its own role in the immune system.

2. Granulysin function: cytotoxicity

2.1 Granulysin as an antimicrobial molecule

The 5-helix bundle structure of granulysin and its structural homology to NK-lysin - which is strongly lytic in nature - suggests a cytotoxic function of this protein[15]. Research has shown that helices 2 and 3 are particularly important in eliciting this function. Wang *et al.* produced peptides from 9 kDa granulysin, and showed that those peptides corresponding to helices 2 and 3 lysed bacteria, while peptides corresponding to helix 3 alone lysed human cells and liposomes, suggesting that different parts of the molecule interact with different target structures[16]. However, as reviewed by Dotiwala and colleagues recently, 9 kDa granulysin is 1000-fold more active in the lysis of bacterial cell membranes as compared to lysis of mammalian cell membranes[11]. Just what causes cytotoxic immune cells to release granulysin

in response to microbes remains to be elucidated, although research has shown that a small lipopeptide present in *Mycobacterium leprae* is able to induce granulysin expression in T cells, suggesting recognition of PAMPs may cause induction of granulysin expression in these cells[17].

Granulysin is involved in the immune response to many different pathogens, including bacteria, parasites, fungi and protozoa. Much research has focussed on the role of granulysin in the immune response to *Mycobacterium tuberculosis*, and a strong correlation between the presence of granulysin and a positive clinical outcome of this disease has been identified[18,19]. Granulysin is capable of killing both extracellular and intracellular *M. tuberculosis*. While extracellular bacteria are targeted directly by granulysin through the ability of this molecule to alter the membrane integrity of a pathogen, increasing membrane permeability and inducing osmotic lysis of the bacterial cell[4,20], the pore-forming molecule perforin is required to allow granulysin to target intracellular bacteria, by providing access into the infected cell[21]. Production of *de novo* 9 kDa granulysin within cytotoxic cells responding to *M. tuberculosis* is contact-dependent, through utilisation of nanotube-like structures produced by the immune cell. Lu *et al.* showed that this contact caused stimulation of signalling pathways involving ERK, JNK and p38 MAPKs within NK cell populations, which led to a 2.2 fold increase in the production of 9 kDa granulysin within these cells[22].

Granulysin has been implicated in the immune response to a number of other pathogens. In addition to *M. tuberculosis*, this molecule is also involved in the immune response to other *Mycobacterium* species, including *Mycobacterium leprae*. Ochoa *et al.* showed that granulysin-expressing $CD4^+$ αβ T cells existed at a 6-fold higher frequency in leprosy lesions of patients with a contained form of the disease as compared to lesions of patients with disseminated disease. These $CD4^+$ αβ T cells were found to target *M. leprae* bacteria through a granule exocytosis-mediated pathway, and granulysin was found to be responsible for the reduced viability of these bacteria[23]. Populations of $CD8^+$ αβ T cells have been shown to use granulysin to kill the fungi *Cryptococcus neoformans*[24], while $V\delta2^+$ γδ T cells expressing granulysin are pivotal in the immune response against the parasite *Plasmodium falciparum*. Farouk *et al.* showed an *in vitro* contact-dependent reduction in viability of these malarial parasites which strongly correlated with granulysin expression[25].

Interestingly, in addition to being involved in the direct killing of pathogen, recent evidence has also suggested that granulysin is required for the delivery of cytotoxic effector molecules

such as granzymes into an infected cell, allowing these serine proteases to kill through initiation of the caspase cascade and apoptosis[26]. Walch *et al.* showed that mice transgenic for human granulysin were better able to clear *Listeria monocytogenes* infections, and postulated that this was due to granulysin increasing the delivery of granzymes into *Listeria*-infected cells[27].

2.2 Additional functions of granulysin

While the role of granulysin as an anti-microbial molecule has perhaps been most extensively studied, this protein also has a number of other functions, which have been reviewed comprehensively by Krensky *et al.*[10]. Granulysin has been identified as a biomarker for transplant rejection and steroid resistance[28], and serum levels of granulysin have also been correlated with severity of graft versus host disease[10]. Granulysin, in particular the 15 kDa isoform, has been associated with a number of skin diseases. In particular, concentrations of up to 5μM 15 kDa granulysin have been observed in the skin lesions and blisters of patients with Stevens-Johnson syndrome, suggesting a cytotoxic capability of this isoform of granulysin[29]. Finally, granulysin has been reported in the early pregnancy decidua, and has been found to be associated with pre-eclampsia[30]. Veljkovic Vujakija *et al.* showed that 85% of uterine NK cells expressed granulysin, and that granulysin expression in decidual lymphocytes was two times higher than that seen in peripheral blood lymphocytes, suggesting a role for this molecule in the control of a broad spectrum of infections at the maternal-fetal interface[31]. Another widely studied role of granulysin is its involvement in the immune response to cancer.

Table 1: Comparison of the two isoforms of human granulysin

	9kDa granulysin	**15kDa granulysin**
Subcellular location	Packaged in cytotoxic granules with granzymes and perforin	Packaged in its own granules
Cellular release	Granule-exocytosis mediated pathway, dependent on calcium	Dependent on PKC, spontaneous secretion
Function	Antimicrobial molecule Cytotoxicity	Immune 'alarmin' Maturation of antigen-presenting cells Migration of immune cells Potential cytotoxicity

3. Role of granulysin in the immune response to cancer

Many studies have demonstrated the involvement of granulysin in the targeting of tumour by cytotoxic immune cells, and show a correlation between the presence of granulysin and a more positive cancer prognosis[32]. For example, higher concentrations of granulysin have been found in gastric carcinoma patients with less advanced cancers (stage II and III) as compared to those patients with more aggressive disease (stage IV)[33]. Additionally, high serum concentrations of granulysin have been associated with a reduced incidence of both hepatic and peritoneal metastases, and a better outcome of curative gastrectomy[33]. Interestingly, recent evidence has shown the ability of NK cells to produce extracellular vesicles containing cytotoxic molecules, including granulysin. When incubated with cancer cells, these vesicles were shown to cause activation of caspase pathways and eventual apoptosis of tumour cells[34].

In addition to these observed correlations between the presence of granulysin and improved outcome, several studies have shown a direct ability of granulysin to kill tumour cells. One experimental method utilised to show this function of granulysin is the use of mice transgenic for human granulysin. Krensky *et al.* generated a mouse transgenic for this molecule, and showed these mice were better able to reject the T cell lymphoma C6VL than their wildtype counterparts[10]. More recently, Hsiao and colleagues produced a humanised mouse model through the irradiation of NSG mice, and subsequent transplantation of human umbilical cord mononuclear cells. Experimentation on this mouse strain showed a reduction of transplanted tumour growth which was facilitated by granulysin-controlled initiation of apoptotic pathways[35]. Furthermore, serum granulysin levels correlated positively with inhibition of tumour growth in these humanised mice[35].

3.1. 9 kDa granulysin kills tumour cells directly

Generally, the 9 kDa isoform of granulysin is thought to be responsible for killing pathogens, and infected or malignant cells due to its cytotoxic nature. The way in which 9 kDa granulysin enters a target cell remains to be elucidated, and a receptor for this molecule has not yet been identified. While it was initially thought that 9 kDa granulysin would enter a target cell *via* pores formed by perforin, recent studies have shown that this may not be the case. Current hypotheses state that 9 kDa granulysin folds in such a manner as to produce a positively charged pocket, which can then interact with negatively charged regions on the infected or transformed cells targeted by cytotoxic immune cells[15,36].

Early work into the mechanism by which 9 kDa granulysin mediated cell death showed that application of recombinant 9 kDa granulysin to the Jurkat T cell line caused an increase in ceramide within these cells, coupled with a concomitant decrease in sphingomyelin[37]. This observation was postulated to be due to the ability of recombinant 9 kDa granulysin to cause activation of sphingomyelinase, which breaks down sphingomyelin into ceramide and phosphorylcholine. Activation of this enzyme would induce pore formation within the sphingomyelin-containing plasma membrane, and hence lysis of the target cell[37]. However, cell death was slow, taking place over 16 hours, and subsequent studies have shown granulysin to induce apoptosis within cells far more rapidly. Additionally, the concentration of granulysin required to kill these cells (50μM) is far higher than has ever been recorded in humans, and as such it is likely a second mechanism of action is being utilised by this molecule to kill target cells[11].

This second mechanism of action of 9 kDa granulysin has been shown to be an increase in intracellular calcium within target cells, and is depicted in figure 1. Studies led by Kaspar *et al.* showed that calcium increase led to mitochondrial damage, an increase in cytochrome c, and eventual activation of caspase 3, initiating apoptosis within the cell[36]. Subsequent research has confirmed this increase in intracellular calcium in response to recombinant 9 kDa granulysin, and has shown the increase to be present in both the cytosol and the mitochondria. Calcium increase was also coupled with a decrease in potassium, and an increase in apoptosis inducing factor; both markers of the initiation of apoptosis[38,39]. Interestingly however, it has been shown that recombinant 9 kDa granulysin and cell-delivered 9 kDa granulysin may actually induce apoptosis through different pathways. Saini *et al.* showed that while recombinant 9 kDa granulysin induced apoptosis in a manner similar to granzymes, through damage to the mitochondria and initiation of caspase 3 as discussed above, 9 kDa granulysin produced and delivered by NK cells caused damage to the endoplasmic reticulum, leading to initiation of apoptosis through the activation of caspases 7 and 12, without activating caspase 3[40]. Researchers postulated that this phenomenon may be due to the lack of additional cytotoxic molecules perforin and granzymes when recombinant 9 kDa granulysin is used, and suggested therefore that recombinant 9 kDa granulysin was compensating for the lack of granzymes in this case[40].

Several studies have shown that the 9 kDa isoform of granulysin is responsible for the killing of tumour cells exposed to this molecule, through mechanisms discussed above. A 2.5 fold increase in DNA fragmentation coupled with a substantial reduction in proliferation was

observed within mouse adenocarcinoma colon 26 cells cultured with recombinant human 9 kDa granulysin, suggesting an initiation of apoptosis[41]. These findings were subsequently corroborated through transfection of athymic nude mice with the human breast adenocarcinoma line MDA-MB-231 or the multiple myeloma line NCl-H929. When directly injected with recombinant 9 kDa granulysin, tumours were seen to cease growing, or indeed were completely eradicated[42]. The apoptosis triggered in these tumour cells in response to 9 kDa granulysin was found to be due to a reduced mitochrondrial membrane potential within the cells, leading to release of AIF and cytochrome c[43].

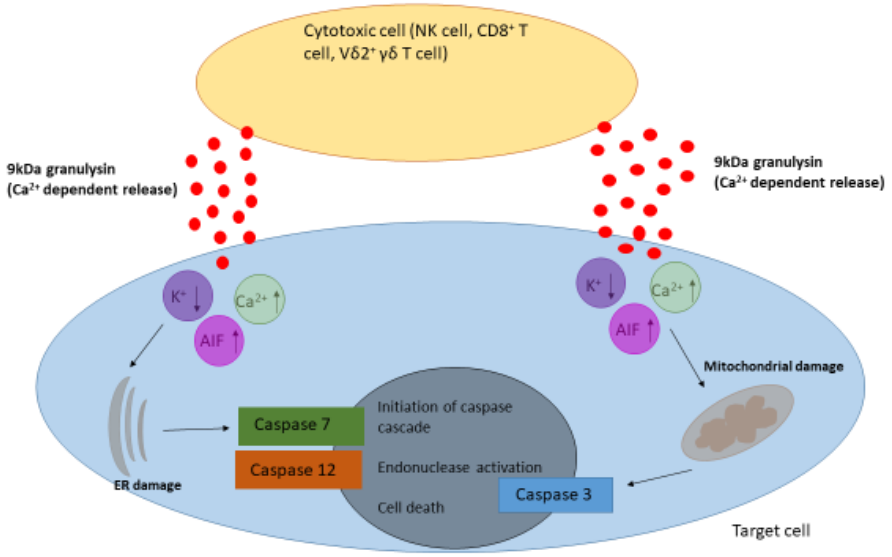

Figure 1: 9kDa granulysin induces target cell death through initiation of the caspase cascade.

On activation, a cytotoxic immune cell releases 9kDa granulysin through a calcium-dependent, granule exocytosis-mediated pathway. Entry of 9kDa granulysin into a target cell causes an increase in the concentration of cytoplasmic calcium, coupled with a simultaneous decrease in cytoplasmice potassium. This results in an increase in apoptosis inducing factor, and subsequent damage to both the endoplasmic reticulum (ER) and the mitochondria. ER damage causes activation of caspases 7 and 12, while mitochondrial damage leads to activation of caspase 3. Induction of the caspase cascade in turn activates endonucleases, which eventually leads to the death of the cell.

3.2 15 kDa granulysin attracts additional immune cell populations

While 9 kDa granulysin has been shown to have broad cytotoxic activity against both infected cells and tumour, the role of 15 kDa granulysin is not as well understood. Initially, this isoform of granulysin was thought to be an inactive precursor; however, research into the 15 kDa molecule of granulysin has shown it may also have a role to play in the immune response to infection and malignancy[10,44]. Evidence shows that 15 kDa granulysin may act as an immune alarmin, defined as any molecule released by a damaged or diseased cell that stimulates an immune response[45]. Alarmins are endogenous mediators, which induce the recruitment and activation of antigen-presenting cells, such as dendritic cells, and promote the generation of the immune response.

Studies investigating the ability of 15 kDa granulysin to act as an immune alarmin have revealed its involvement in both the maturation and migration of dendritic cell populations, and have been extensively reviewed by Zitvogel *et al.*[44]. The 2 major functions of 15 kDa granulysin are portrayed in figure 2. Recombinant 15 kDa granulysin has been shown to cause the differentiation of monocytes to dendritic cells, in addition to the maturation of populations of immature dendritic cells. Research by Clayberger *et al.* showed that in response to culture with 10nM recombinant 15 kDa granulysin, populations of human $CD14^+$ monocytes were observed to increase expression of CD80, CD83 and CD86, markers of dendritic cell differentiation[12]. This finding was corroborated by Castiello and colleagues, who showed that culture of human monocytes with 10nM recombinant 15 kDa granulysin caused an increase in genes relating to immune response, chemotaxis and cell adhesion within these monocytes. Furthermore, evidence was shown for the activation of pathways related to fundamental dendritic cell function within cultured monocytes, including pathways for T cell activation and Th1 development[46]. Clayberger *et al.* also showed the ability of recombinant 15 kDa granulysin to cause maturation of human immature dendritic cells. When 10nM recombinant 15 kDa granulysin was added to cultures of immature dendritic cells, an increase in expression of CD80, CD86, HLA-DR and CD40 was observed, indicative of maturation[12]. These findings provide evidence for the dual ability of granulysin within the immune system; in addition to killing cells directly through release of 9 kDa granulysin, cytotoxic immune cells also release 15 kDa granulysin in order to induce the maturation of dendritic cells. The maturation of dendritic cells in response to infection or tumour is crucial for the additional activation of cells of the adaptive immune response to the pathogen or malignancy, and therefore initiation of both arms of the immune system.

15 kDa granulysin has also been shown to cause the migration of several immune cell populations, including dendritic cells. Studies by Deng *et al.* reported migration of populations of memory $CD4^+$ and $CD8^+$ αβ T cells, monocytes, NK cells, and mature, but not immature, dendritic cells in response to 10nM recombinant 15 kDa granulysin[47]. Later studies by Tewary and colleagues also observed migration of immature dendritic cells in response to both 10nM recombinant 15 kDa granulysin and granulysin-containing supernatants taken from cultures of both primary NK cells and HuT78 T cell lymphoma cell lines transfected with 15 kDa granulysin[45]. When 15 kDa granulysin present within supernatants was inhibited through use of a blocking antibody specific for this molecule, migration of immune cell populations was diminished, suggesting 15 kDa granulysin to be responsible for the migration seen[45]. Further studies by Tewary *et al.* showed that application of 15 kDa granulysin to immature dendritic cells increased the ability of these cells to cause proliferation of allogeneic T cells. Granulysin-treated dendritic cells were able to stimulate proliferation of T cells at a dendritic cell:T cell ratio of 1:2500, while dendritic cells which were not pre-treated with granulysin required a ratio of 1:50 to induce T cell proliferation[45].

A question that remains to be answered however is what 15kDa granulysin uses as a receptor, and how it can bind to dendritic cells in order to cause their migration and maturation. Through observations made in previous studies, it appears that many of the effects elicited by alarmins seem to be mediated by G protein coupled receptors. In fact, both Deng *et al.* and Tewary and colleagues showed that the addition of pertussis toxin to immature dendritic cells prior to treatment with recombinant granulysin abolished the migratory capacity of this molecule[45,47]. Therefore, it can be postulated that granulysin may bind a G protein coupled receptor.

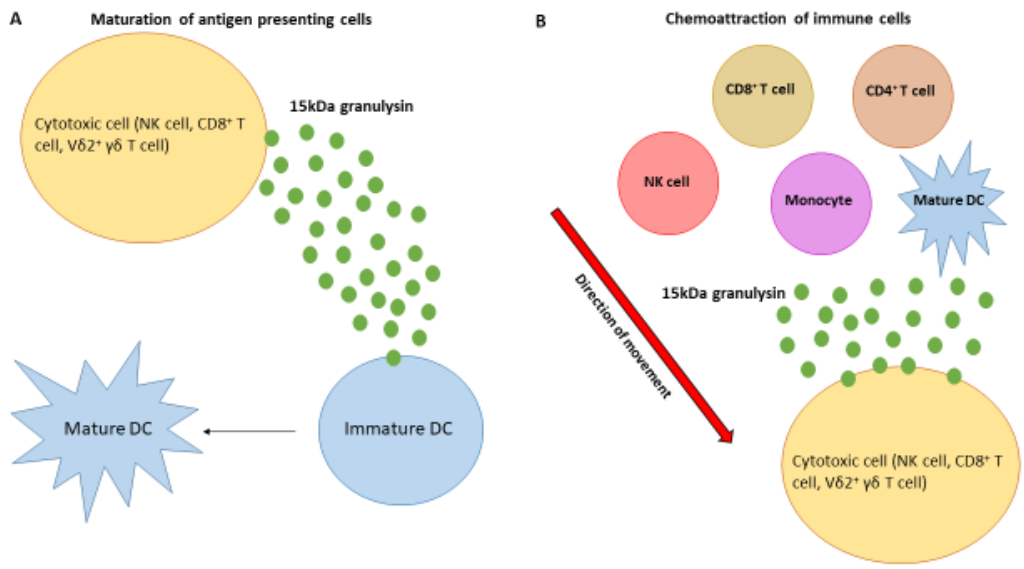

Figure 2: 15kDa granulysin functions as an immune 'alarmin'.

15kDa granulysin has been shown to function as an immune 'alarmin', due to findings which show this isoform to be capable of induction of the recruitment and activation of antigen-presenting cells, such as dendritic cells, and promote the generation of the immune response. Panel A depicts the role of 15kDa granulysin in the maturation of immature dendritic cells, while panel B shows release of 15kDa granulysin by a cytotoxic cell subsequently inducing the recruitment of additional immune cell populations to a site of infection or malignancy.

The observation made by Deng *et al.* that mature but not immature dendritic cells could migrate in response to recombinant 15 kDa granulysin is contrary to what might be expected, as it would seem that there is little benefit in the recruitment of an already mature dendritic cell to a site of inflammation. However, studies carried out by our own research group have added to these findings. We found that 150ng/ml (10nM) recombinant 15 kDa granulysin induced the migration of mature, but not immature, dendritic cells. However, a higher concentration of 1µg/ml (66nM) recombinant 15 kDa granulysin was sufficient to initiate the migration of immature dendritic cells. Interestingly, mature dendritic cells actually appeared to migrate away from this higher concentration of recombinant 15 kDa granulysin, indicative of chemorepulsion, or fugetaxis (derived from the Latin words *fugere*, to flee from; and *taxis*, movement) of these cells (Sparrow *et al.*, submitted). Similarly, when we repeated these experiments using supernatants from the co-culture of anti-tumour Vδ2$^+$ γδ T cells with B cell lymphoma Daudi or Raji cell lines, which contained granulysin, we found that those supernatants containing high concentrations of granulysin caused the migration of immature, but not mature, dendritic cells, in addition to the fugetaxis of mature dendritic cells.

Conversely, supernatants containing low concentrations of granulysin initiated the migration of mature, but not immature, dendritic cells.

This ability of 15 kDa granulysin to cause both the chemotaxis and fugetaxis of mature dendritic cells, dependent on concentration, is particularly interesting. Several instances of the dual capacity of chemoattractants to cause both the attraction and repulsion of the same immune cells, dependent on concentration, have been reported. IL-8 has been shown to be a potent chemoattractant for neutrophil populations; however, recent evidence has shown neutrophils to also migrate away from IL-8. Whether a neutrophil migrated towards or away from IL-8 was dependent on the absolute concentration of this molecule[48]. Using microfluidic linear gradient generators and time-lapse microscopy, results reported by Tharp *et al.* showed that at concentrations of 120nM, neutrophils were seen to migrate towards IL-8, while at concentrations of 1.2μM, neutrophils displayed potent fugetaxis[48]. Other chemokines reported to cause both chemotaxis and fugetaxis of immune cells include CCL19 and CXCR3 ligands. Malet-Engra *et al.* showed that while CCL19 acted as a potent B cell chemoattractant, small populations of B cells were also seen to migrate away from high concentrations of this chemokine[49], while populations of dendritic cells were observed to migrate away from CXCR3 ligands, if these ligands were immoblised[50]. A similar phenomenon has been reported for the ability of stromal cell derived factor (SDF)-1 to cause the attraction and repulsion of T cells. At 100ng/ml, SDF-1 caused chemoattraction of both naïve and memory $CD4^+$ and $CD8^+$ αβ T cells, while higher concentrations of 10μg/ml caused repulsion of these cells[51].

Experiments carried out by our own research group suggest that granulysin is acting in a similar manner. While 150ng/ml recombinant granulysin and supernatants containing low concentrations of granulysin caused the attraction of mature dendritic cells, 1μg/ml recombinant granulysin, and supernatants containing high concentrations of granulysin caused the repulsion of these cells (Sparrow *et al.,* submitted). While it has been shown that the serum concentration of granulysin within healthy individuals is approximately 9ng/ml[11], which is substantially lower than the concentrations used here, concentrations as high as 75μg/ml have been reported in the blister fluid of Stevens-Johnson syndrome patients[29]. This suggests that higher concentrations of 15 kDa granulysin are possible in sites of inflammation, such as in response to infection or cancer. We therefore hypothesise that granulysin acts on both immature and mature dendritic cells in ways that increase the ability of these cells to carry out their roles within the immune system - recruiting immature

dendritic cells to the site of inflammation and then driving mature dendritic cells to the local lymph node to stimulate the adaptive immune response.

4. Concluding remarks: The two sides of granulysin

Granulysin, initially thought of as an antimicrobial peptide with cytotoxic effects against both pathogen, and infected or transformed cells, may have a considerably greater role in the immune response to tumour than first thought. While the 9 kDa isoform is broadly cytotoxic and has been shown to be capable of the direct killing of tumour cells, the 15 kDa isoform has recently been implicated in the migration and maturation of additional immune cell populations to the site of tumour. Therefore, it can be postulated that granulysin is involved in the immune response to tumour twofold, and this hypothesis is shown in figure 3. Cytotoxic cells of the innate immune system, such as NK cells, $V\delta2^+$ γδ T cells and CD-1 restricted innate immune cells respond rapidly to tumour, and release 9 kDa granulysin following activation in response to target *via* a granule exocytosis-mediated pathway. Subsequently, 9 kDa granulysin enters the target cell to initiate apoptosis. At the same time, 15 kDa granulysin is constitutively released by these innate cytotoxic cells, and may assist in bringing additional immune cell populations, including dendritic cells, to the site of a tumour. Once at a tumour site, immature dendritic cells will endocytose tumour material and then, with a contribution from granulysin, mature. Once matured, these cells will be repelled by the high concentrations of granulysin present at the tumour site, and migrate away from this location. Here, they will be able to pick up the chemokine gradients of CCL19 and CCL21, allowing them passage to the lymph nodes, in order to activate cells of the adaptive immune response. These observations open up various possibilities of therapeutically using both isoforms of granulysin in combination, in order to increase both the immediate immune response to tumour, in addition to initiation of the adaptive immune response.

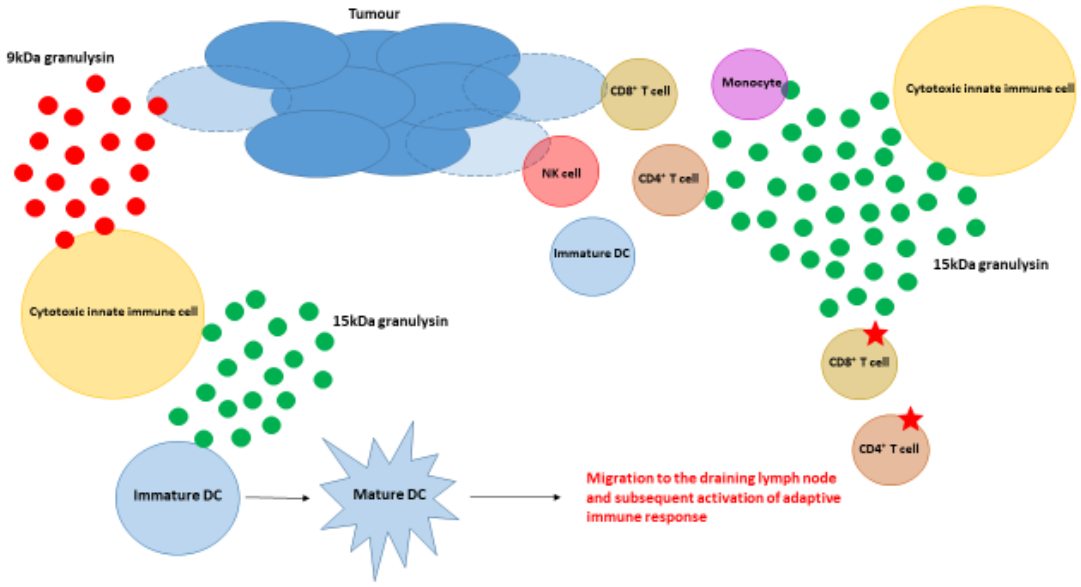

Figure 3: Proposed mechanism of action of the two isoforms of granulysin in response to cancer

Cytotoxic innate immune cells, including NK cells, CD1-restricted cells and Vδ2$^+$ γδ T cells are brought to a tumour target, due to the release of inflammatory chemokines from the tumour cells. Once at the site of tumour, these cells are activated by tumour antigen, and subsequently release 9kDa granulysin through a calcium-dependent exocytosis-mediated pathway. 9kDa granulysin enters the cell, and elicits its cytotoxic function through endoplasmic reticulum and mitochondrial damage, dysregulation of calcium and potassium ions, and eventual initiation of the caspase cascade. 15kDa granulysin is also released in response to activation of innate immune cells in response to tumour. This isoform acts two-fold; it causes the migration of additional immune cells, increasing the initial immune response to the tumour. Additionally, 15kDa contributes to the maturation of immature dendritic cells at the tumour site. Following maturation, dendritic cells migrate to the draining lymph node, activating circulating CD8$^+$ and CD4$^+$ T cells, and initiating the adaptive immune response to the tumour.

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