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Human granzymes: Related but far apart

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ABSTRACT

Granzymes (GZMs) are a class of serine protease, found in cytoplasmic granules of cytotoxic T cells and natural killer cells. The main function of these proteins has been recognized as wiping out viral infections via inducing the apoptosis.

This review will highlight inter and intra species differences of GZMs in terms of their functional and structure. These futures may help to device a strategy for isolation of human specific GZMs, which are needed for understating of their role in immune system and devising an effective immune therapy.

Preface on granzymes

Exocytosis requires direct contact between the cytotoxic lymphocytes (CL) and target cell. The granule exocytosis (release of granule contents) is performed by a set of proteases named granzymes (GZMs). GZMs make up a major portion of CL lytic granules. They are responsible for the majority of cell death induced by CLs [1–3]. In this step, the CLs polarize and reorient their granules toward the target followed by their release into the cleft between the CL and infected cell, also known as the "conjugation step". After translocation of these granules, like GZMs, into the target cytosol, they cleave various substrates leading to target cell death (apoptosis) [4–6]. This quick (5–30 min) and irreversible step is considered as a "lethal hit". In this battle, CLs are comparatively resistant to GZMs. They remain intact and can repeat this process with additional targets [2–5].

The term "granzyme" was first given to these "granule-enzymes" by Masson and Tschopp in 1987 [7]. Since then a total of 12 GZMs have been discovered in various species, of which 5 are found in human. They are part of a family of related serine proteases (EC 3.4.21) where serine serves as the nucleophilic amino acid at the enzyme's active site [8]. While the majority of GZMs seem to exert their effect locally by crossing the immunological synapse (from the effector cell to the target cell) there are also soluble GZMs found in the extracellular fluid [9–11]. Though the biological effects of these soluble GZMs are not clear, it has been proposed that they may contribute to the degradation of extracellular matrices such as cartilage, as seen in rheumatoid arthritis (RA), and have antibacterial and antiviral activities [12–15].

Reliable detection of GZMs remains a challenge, due to the considerable similarities between them. For example, anti-GZMB antibodies (Abs) strongly cross-react with GZMA, -G, and -H, while anti-GZMD Ab cross-react with GZMC, -F, and -K [7,16]. Furthermore all GZMs maintain certain essential structural characteristics such as a signal peptide, a dipeptide that is cleaved from the pro-enzyme during activation. It is critical to isolate antibodies that are not only specific to each type but also could distinguish between their active and inactive forms. Different aspects of the GZMs have been covered by several excellent reviews [17,18]. For that reason this review will focus on the protein structure of GZMs and highlight their similarities in action and structure.

The GZM family

Twelve different GZMs; A, B, C, D, E, F, G, H, J, K, M, and N have been identified so far. With the exception of GZMJ that is unique to rat, others can be found in more than one species. For instance GZMA, -B, -H, -K, and -M have been identified in humans but are not unique to our species [19–23]. They are also conserved in related species, such as chimpanzee, rhesus monkey and other

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Table 1
Protein and genomic sequence identity of *H. sapiens* granzymes versus their orthologs.

Species	Identity (%) with *H. sapiens*									
	GZMA		GZMB		GZMH		GZMK		GZMM	
	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA
P. troglodytes	98.1	99.2	98	98.7	94.7	98.1	99.2	99.6	–	–
M. mulatta	91.6	95.2	86.2	92.8	–	–	93.6	95.6	89.7	92.1
C. lupus	73.5	81.7	70.7	77	74.4	82	72.3	80.6	–	–
B. taurus	73.4	82.8	73	79.6	77.2	81.3	72.7	81.3	70.9	77.2
M. musculus	68.8	76.7	68.6	75.9	60.7	69.3	73.3	78.3	70.6	74.1
R. norvegicus	70.5	77.9	69.5	76.4	–	–	73.4	78.4	69.8	73.9
G. gallus	54	61.9	–	–	–	–	–	–	52.1	62.9
D. rerio	–	–	–	–	–	–	40.9	52.3	–	–

Dash line represent data not found.

mammals including dog, cow, mouse, rat, but also in chicken and even in zebrafish (Table 1). Human GZMs have the largest homology with those of *Pan troglodytes* (common chimpanzee) and *Macaca mulatta* (rhesus monkey), sharing over 90% sequence identity at the protein level (Table 1). However, since the names are assigned based on the signal homology and not role, it remains to be seen if these orthologous enzymes also share the same biological function. The similarities within the 5 human GZMs can is depicted in a rooted phylogenetic tree (Figs. 1 and 2) displaying the evolutionary relationship between the 5 human GZMs. Except GZMM paralog that has the highest distance from the rest in its amino acid composition, the remaining 4 can be grouped into two sets. GZMB and GZMH, displaying the highest sequence similarity of 71% (100% certainty) and GZMA and -K that have similarity of 39.7% (with 87% certainty) (Fig. 2). These sequence similarities do not necessarily implies a similarity at functional level, as GZMs vary in their substrate specificities [24]. Human and mouse GZMB orthologs, for instance, share over 86% identity at the protein levels but differ in their substrate specificity [25]. On the other hand the human GZMA and B paralogs differ from each other in their sequences but recognize the same substrate and in some cases cleave them at the same site [26].

The human GZMs genes are located on 3 different chromosomes and are similar in size and share similar domains. They fall into three gene clusters: tryptases, chymases and met-ases. Their grouping on the evolutionary tree and position on the chromosomes hints a gene duplication of GZMA and -K and of GZMB and -H. GZMA and -K are members of the tryptase family. They are trypsin-like serine proteases, and are found in the tryptase cluster on chromosome five [24]. GZMB and -H on the other hand are part of chymase cluster and are found on chromosome 14, which also encodes the gene for cathepsin G (CG), mast cell chymase-1 (CMA1) and other chymotrypsin-like serine proteases [27]. GZMM has the farthest distance on the evolutionary tree from other 4 human GZMs, is in met-ase cluster located on chromosome 19 [24]. It has been suggested that under selective pressure, mechanisms of duplication, recombination, and mutation are responsible for boosting the GZMs repertoire and diversity [28].

All human GZMs are present as a monomer except GZMA that forms a dimer [29]. GZMs have a remarkably similar catalytic triad in their active site. It is composed of His57, Asp102 and Ser195 (Fig. 2, Table 2 indicated in orange). These enzymes are first translated as an inactive form (zymogen) and are then converted into an active form upon the cleavage of a dipeptide during activation. A direct comparison between the inactive (zymogen) and active GZMs were not possible, since crystal structures for both forms are not available. The crystal structure available are of either inactive (GZMK) or active form (GZMA, -B, -H and -M), but not both. For that reason, crystal structure of inactive (zymogen) GZMK was compared to active form of GZMA to gain an insight into the activation process of the enzyme (Table 3). Comparing GZMK zymogen to active GZMA, its closest analog (Fig. 1), exposes a major structural difference. The absence of enzymatic activity in the zymogen is the active site conformation: the distance between Ser195 and Asp102 in pro-GZMK (5.6 Å) is too large for these amino acids to interact with the substrates in the catalytic triad. However, once the two amino acid of the pro-peptide (Fig. 2, in red) is cleaved from the N-terminus by cathepsin C, the N-terminal tail is left with two hydrophobic isoleucines and flexible glycines (IIGG) to fold and docks into a hydrophobic region of zymogen. This results in an allosteric conformational change that changes the His57 spatial position and decreasing the distance between Ser195 and Asp102 to 2.5 Å to form the triad that effectuates target protein cleavage (Table 3).

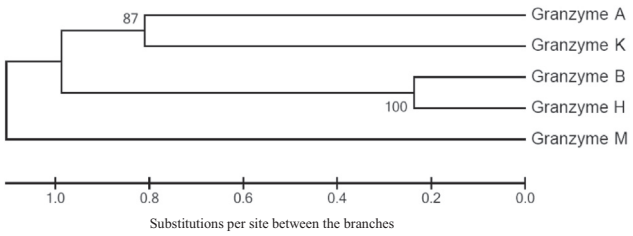

Fig. 1. Evolutionary tree for 5 human granzymes. The UPGMA method was used to generate the tree. Similar tree topologies were obtained using Neighbor-Joining, Maximum Likelihood and Maximum Parsimony methods. Numbers on the branches (*Bootstrap values*) indicate percent certainty of branch topology. Scale at bottom indicates the number of substitutions per site between the branches. The program MEGA v.5.22 was used to generate all trees. Human GZMA, -B, -H, -K and -M pro-enzyme sequences were obtained from UniProt.org (entry numbers P12544, P10144, P20718, P49863 & P51124, respectively).

Natural GZM substrates

The exact definition of GZM substrates is still under debate because many aspects of their protease/substrate biology remain to be solved [26]. Thus far, in addition to extracellular proteins of target cells, different intracellular proteins have been identified as their substrates [15,26,30–33]. Fibronectin, laminin, vitronectin, basement membranes, collagen type IV, and cartilage proteoglycan are examples of extracellular proteins processed by GZMB. To date, at least 500 substrates have been described for GZMB, encompassing a broad spectrum of structurally and functionally different protein classes. For other GZMs, nuclear, cytoskeletal, membrane receptor and viral proteins are found to be among their substrates. As previously stated, GZMA and GZMK are tryptases that cleave after basic amino acid residues (designated the "P1 residue") in target proteins [18]. However, recent studies show that some

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