A rat gene homologous to human granule membrane protein 17 is expressed by natural killer cells, CD8⁺ T cells, and a mast cell line

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The human granule membrane protein of 17000 Mᵋ (GMP-17), formerly called p15-TIA-1, localizes to the membranes of cytotoxic granules of natural killer (NK) cells and translocates to the plasma membrane upon degranulation and target cell lysis (Medley et al. 1996). The amino acid sequence of GMP-17 is identical to those predicted by two human cDNA clones previously isolated from NK cells [NKG7 (Turman et al. 1993)] and mononuclear cells from a chronic myelogeneous leukemia patient [GIG-1 (Shimane et al. 1994)], respectively.

As a result of random characterization of several clones obtained from a rat NK-cell cDNA library, we isolated a cDNA (GenBank accession number AF082535) with 73.3% nucleotide identity and 71.5% amino acid identity to human GMP-17/NKG7/GIG-1 (Medley et al. 1996; Shimane et al. 1994; Turman et al. 1993). Although the rat GMP-17 cDNA predicts a protein similar to human GIG-1 throughout, it differs considerably in its C-terminal half from the first reported human NKG7 sequence (Turman et al. 1993). The latter has later been altered after re-evaluation of the material (GenBank accession number U09608), and brought to be identical to GIG-1 (Shimane et al. 1994) except for one nucleotide (silent mutation).

The full-length clone contains a 5’ untranslational region of 144 nucleotides (including an in frame stop codon upstream of the initiation codon) and a 3’ untranslational region of 171 nucleotides. Although the -3 to +4 sequence of the putative initiation codon (TCCATG) lacks the important purine in position -3 of the Kozak consensus sequence (Kozak 1986), its similarity to the human GMP-17 start codon (CCCATG) plus the lack of alternative sites upstream or downstream indicates that it is the translation initiation site of rat GMP-17.

The predicted putative protein is a type III integral transmembrane protein of 165 amino acids, with a relative molecular mass of 17700 Mᵋ. Amino acids 1-22 meet the requirements for a leader sequence including a putative signal sequence cleavage site between residues 22 and 23 (von Heijne 1986), suggesting that the mature GMP-17 protein could exist as a 143-residue, 15300 Mᵋ molecule, with three hydrophobic domains rather than four. However, peptide sequencing of the first 25 N-terminal amino acids of human GMP-17, purified using a monoclonal antibody directed against its C-terminal end, indicated that the human protein is not cleaved (Medley et al. 1996). Thus, the mature rat GMP-17 protein most likely contains four hydrophobic, possibly membrane-spanning, regions (Fig. 1A, B). A possible site for N-linked glycosylation is present at residue 35-37. Notably, both rat and human GMP-17 contain an acidic aspartic acid residue within the first hydrophobic domain (Shimane et al. 1994; Turman et al. 1993), suggesting that GMP-17 associates with other membrane proteins. The putatively intracytoplasmic C-terminal ends of rat and human GMP-17 contain the sequence YETL. As no additional YxxL motifs are conserved, it does not meet the criteria for the immunoreceptor tyrosine-based activation motif (ITAM) YxxLX₆₋₈YxxL (Reth 1989).

Sequence comparison with available protein database sequences revealed similarity to other putative four-transmembrane-region proteins, such as the lens fiber membrane proteins MP19 and MP20 (Gutekunst et al. 1990; Kumar et al. 1993). However, neither rat nor human GMP-17 share apparent similarity to the transmembrane 4 superfamily (Wright and Tomlinson 1994).
Amino acid sequence of rat GMP-17 compared with human GMP-17. Hydrophobic domains are underlined, and an arrowhead indicates a possible signal sequence cleavage site. Identical amino acids are indicated by dashes, and stop codons are represented by asterisks. The cDNA clone was sequenced at both strands using cyclic sequencing and automatic sequencing equipment. Software supplied through the Norwegian EMBNet node at the Biotechnology Centre in Oslo was used for computer analysis of sequences.

Hydropathicity profile of the predicted rat GMP-17 peptide performed by the method of Kyte and Doolittle (Kyte and Doolittle 1982). The numbers on the horizontal axis designate the amino acid residue positions.

Southern blot analysis of genomic DNA from DA and PVG rats, digested with 36 different restriction endonucleases and hybridized to a rat GMP-17 full-length cDNA probe, showed a simple pattern with few bands. This suggests that the rat gene for GMP-17 [named Nkg7 after the first publication of the human gene (Turman et al. 1993)] is a single gene with no close relatives (data not shown). Two restriction fragment length polymorphisms were found with the enzymes Sty I and Tth 111I, respectively. The latter was used as a marker for Nkg7 in a linkage analysis of a panel of 218 (DA × PVG) F1 × DA rats. Nkg7 cosegregated with D1Rat27 (7 crossovers) and D1Rat222 (11 crossovers), localizing Nkg7 to RNO1, between the markers D1Rat27 and D1Rat222 (data not shown). The map distances were: D1Rat222 – 5.0 cM (LOD 47.0) - Nkg7 – 3.2 cM (LOD 53.0) - D1Rat27 – 0.9 cM (LOD 61.9) - D1Rat224 (Fig. 2). A further 11.9 cM (LOD 32.0) from D1Rat224 we located a rat leukocyte receptor gene complex containing two new genes encoding immunoglobulin like receptors expressed selectively by NK cells and neutrophilic granulocytes, respectively (Berg et al. 1999). This chromosomal region is linked to the loci Cea (Ding et al. 1996) and Cyp2b (Rampersaud and Walz 1987; Yamada et al. 1994), whose human counterparts map to HSA19q13.2–13.4 (Miles et al. 1988; Mohrenweiser et al. 1991). In accordance, human NKG7 is also located on HSA19 (Turman et al. 1993), suggesting conservation and a high level of similarity between these chromosomal regions.

By northern blot analysis, a transcript of about 0.9 kilobases was present in interleukin 2 (IL-2) activated NK cells and the NK cell lines A181 and RNK-16 (Fig. 3). IL-2-activated NK cells from the rat strains AO, AGUS, AUG, DA, LEW, and PVG contained similar levels of mRNA (data not shown). Whereas no transcription was detectable in CD4+ thoracic duct T cells or B cells even after prolonged exposure (not shown), a weak signal was detected in resting CD8+ thoracic duct T cells and a more pronounced signal in concanavalin A (ConA) blasts (the latter is most likely accounted for by the CD8+ T-cell subset, although we cannot exclude a contribution from CD4+ T cells). The lane containing RNA from spleen gave a weak signal, and there were no detectable bands in the other tissues tested (Fig. 3). Our observations are in accordance with

Fig. 2. Genetic linkage map of the region on rat Chromosome 1 containing Nkg7. Map units are shown in centiMorgans. A backcross panel [(DA × PVG) F1 × DA rats] was typed at the anonymous loci D1Rat27, D1Rat222, and D1Rat224 (Whitehead Institute/MIT Center for Genome Research, Rat Genomic Mapping Project) (http://waldo.wi.mit.edu/rat/public/) by simple sequence length polymorphism analysis using specific primer pairs (Research Genetics, Huntsville, Ala.) in polymerase chain reaction, followed by horizontal gel electrophoresis (Metaphor agarose, FMC BioProducts, Rockland, Me.) and visualization by ethidium bromide staining. Linkage analysis was performed using the MAPMAKER/EXP computer package (Lander et al. 1987). Map distances were calculated from recombination frequencies according to the Kosambi linkage function (Green 1981)