Supplemental Data

Involvement of MITF-A, an alternative isoform of *mi* transcription factor, on the expression of tryptase gene in human mast cells

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Results

Expression of MITF-A and tryptase-beta1 in the human mast cells

We examined the mRNA expression of MITF isoforms in the human CD34+ progenitor derived mast cells (hMCs) and HMC-1 cells using the specific primers shown in Supplemental Data Table S1. Total RNAs extracted from hMCs and HMC-1 cells were used to synthesize cDNA by RT-PCR. Both hMCs and HMC-1 cells strongly expressed the transcript of MITF-A. The transcripts of MITF-E and -H were expressed weakly in HMC-1 cells, but not detected in hMCs (Figure 1A). Several tryptase genes are clustered on chromosome 16p13.3. Tryptase-beta is the main trypsin-like serine proteases expressed in mast cells, whereas tryptase-alpha predominate in basophiles (Galli, 2000). We thus examined the expression of tryptase-beta in the human mast cells, HMC-1 cells and the hMCs, by RT-PCR. The transcript of tryptase-beta1 was remarkably expressed in both hMCs and HMC-1 cells, but tryptase-beta2, a alterative splicing isoform, was not detected (Figure 1B). In addition, tryptase beta1 was negative in 293 cells, non-mast cell line. The expression level of tryptase-beta1 mRNA in hMCs was higher than that in HMC-1 cells.

Methods

Reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted from the cells using Trizol reagent (Invitrogen, Carlsbad, CA). For cDNA synthesis, RNA was reverse-transcribed with the superscript One-Step RT-PCR kit (Invitrogen) for 1 h at 42oC according the manufacturer's instruction. To examine the expression levels of the genes in the human mast cells, PCR was done with the platinum high fidelity Taq DNA polymerase system using a pair of primers as shown in Supplemental Data Table S1. PCR amplification was performed as follows: denaturation (94oC, 30 s), annealing (58oC, 30 s), and extension (72oC, 50 s). The PCR products were electrophoresed on 1.2% agarose gel in 1×TAE buffer and photographed on UV lamp.

Table S1. **List of the specific primers of genes used for PCR amplication.**

Genes	Primer Sequence	Products
5'-Primers		
MITF-A	5'-TGAAGAGCCCAAAACCTATTACGA-3'	791 bp
MITF-C	5'-CTTCAGTGGTTTTCCCACGAGCT-3'	742 bp
MITF-E	5'-AGTAGCAGGGGTTAGTAGGTGGAT-3'	744 bp
MITF-H	5'-GGAGGCGCTTAGAGTTCAGATG-3'	722 bp
MITF-D	5'-GTTTTAACCTGACAGGCTTTGAATA-3'	687 bp
MITF-B	5'-CCAAAGTGCAAACGAAGGGTCTCA-3'	438 bp
MITF-M	5'-CCTTCTCTTTGCCAGTCCATCTTC-3'	555 bp
MITF-J	5'-CTCTCCATGAGTCTGAGCATCTAA-3'	647 bp
Tryptase-beta1	5'-CCAGGATGCTGAATCTGCTGC-3'	840 bp
Tryptase-beta2	5'-CCAGGATGCTCCTCCTTGCTC-3'	861bp
GAPDH	5'-gaaggtgaaggtcggagt-3'	226 bp
3'-Primers		
MITF-com	5'-GATCAATCAAGTTTCCCGAGACAG-3'	
Tryptase-beta	5'-GCCTACTCACGGCTTTTTGGGGA-3'	
GAPDH	5'-gaagatggtgatgggatttc-3'	