Immunogenetics (2000) 51:246–248 © Springer-Verlag 2000

BRIEF COMMUNICATION

Zihai Li · Veronika Groh · Roland K. Strong
Thomas Spies

A single amino acid substitution causes loss of expression of a *MICA* allele

Received: 4 August 1999 / Revised: 27 September 1999

Key words MHC · *MICA* · Tumor cell lines · Null allele

The human major histocompatibility complex (MHC) class I homologues MICA and MICB are stress-inducible surface molecules that are not associated with β_2-microglobulin and peptides, are expressed in intestinal epithelium and epithelial tumors, and are recognized by $V_\delta 1$ $\gamma\delta$ T cells, a subset of $\gamma\delta$ T cells that is enriched in epithelial sites (Bahram et al. 1994; Groh et al. 1996, 1998, 1999). Analogous to the interactions of MHC class I molecules with inhibitory or activating natural killer (NK) cell receptors that are expressed on NK cells and T cells (Lanier 1998), MICA/B are ligands for an activating receptor, NKG2D, which is expressed on most NK cells, $CD8^+$ $\alpha\beta$ T cells and $\gamma\delta$ T cells (Bauer et al. 1999).

MICA/B are variably expressed in some lung, breast, kidney, ovary, prostate, and colon carcinomas (Groh et al. 1999). While exploring this heterogeneity using cell lines, we identified two gastric epithelial tumor lines that lacked expression of MICA protein despite the presence of abundant mRNA. Analysis of this deficiency showed that both cell lines express a single allele of *MICA* (*MICA010*), which is defective because of a single amino acid substitution in the $\alpha 1$ domain that can be predicted to interfere with a stable protein fold.

Epithelial tumor cell lines were screened by flow cytometry for lack of expression of MICA and/or MICB, using monoclonal antibodies (mAbs) specific for MICA (mAbs 2C10 and 3H5) or MICA and MICB (mAbs 6D4 and 6D5) (Groh et al. 1996, 1998). Two gastric tumor cell lines, AGS and KATO III, had little or no surface MICA, compared with the positive control HCT116 and Lovo colon carcinoma lines, although they contained equal or larger amounts of *MICA* mRNA. AGS cells expressed *MICB* mRNA and stained with the mAbs 6D4 and 6D5, whereas KATO III cells had little or no *MICB* mRNA (Fig. 1A,B; all cell lines were from the American Type Culture Collection). We examined whether the lack of MICA surface expression was due to a defect in protein processing in the secretory pathway. Immunoprecipitation with the mAbs 2C10 and 3H5 from lysates of metabolically labeled cells, *N*-glycanase treatment of immunocomplexes, and sodium docecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) detected the 43,000 M_r MICA polypeptide in HCT116 but not in AGS and KATO III cells (Fig. 1C). However, transfection of cDNA or of a cosmid encoding *MICA* (allele *004*) restored surface MICA on KATO III cells (Fig. 2A). This indicated that expression of the endogenous gene was not impaired by a post-transcriptional or protein-processing defect, but was likely caused by a deleterious sequence alteration.

A substantial number of allelic variants of *MICA* have been described (Fodil et al. 1996), which have so far not been associated with differences in function. Neither the distribution of amino acid substitutions nor the crystal structure of MICA have offered potential explanations (Li et al. 1999). Using specific primers and reverse transcription-polymerase chain reaction, *MICA* mRNA sequences were derived from KATO III and AGS cells. Direct sequencing of amplicons identified a single sequence that was identical in both cell lines and corresponded to the *MICA010* allele, which has a proline for arginine substitution at position 6 in the first β strand of the $\alpha 1$ domain (Fig. 2B) (Fodil et al. 1996). Arginine at this position is conserved in all *MICA* and *MICB* alleles, as well as in homologous sequences from diverse nonhuman primate species (Ando et al. 1997;

Z. Li[1] · V. Groh · R.K. Strong · T. Spies (✉)
Fred Hutchinson Cancer Research Center,
1100 Fairview Ave. N., Seattle, WA 98109, USA
e-mail: tspies@fred.fhcrc.org
Tel.: +1-206-6676940
Fax: +1-206-6675978

Present address:
[1] Center for Immunotherapy, University of Connecticut Health Center, MC 1601, Farmington, CT 06030, USA

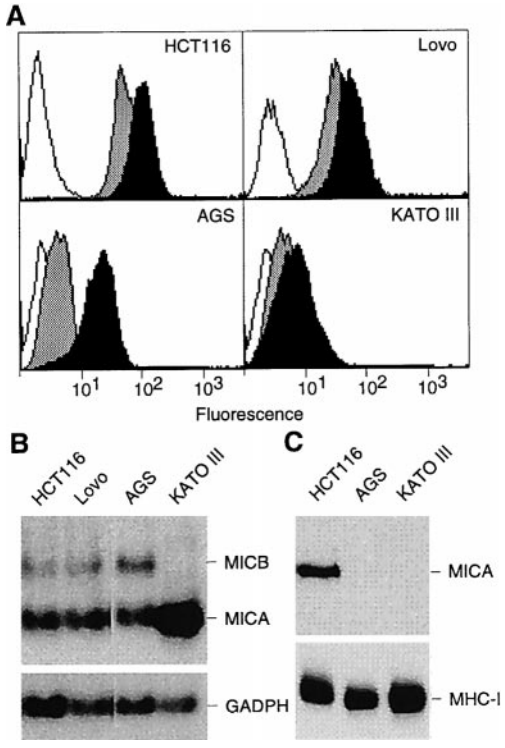

Fig. 1A–C Loss of MICA expression in KATO III and AGS cells. **A** Flow cytometry of HCT116, Lovo, AGS, and KATO III cells using monoclonal antibody (mAb) 2C10 (anti-MICA, *gray profiles*) and mAb 6D4 (anti-MICA and -MICB, *black profiles*). *Open profiles* are immunoglobulin isotype control stainings. **B** By blot hybridization, *MICA* mRNA was expressed in AGS and KATO III cells (*upper panel*). Lower panel shows glyceraldehyde 3-phosphate dehydrogenase (*GADPH*) control mRNA. **C** Immunoprecipitation and SDS-PAGE failed to detect MICA protein in [S^{35}]-methionine-labeled AGS and KATO III cells (Groh et al. 1996). *Bottom panel* shows control major histocompatibility complex class I (*MHC-I*) precipitations using mAb W6/32 (Parham et al. 1979). Immunocomplexes were treated with poly *N*-glycanase F

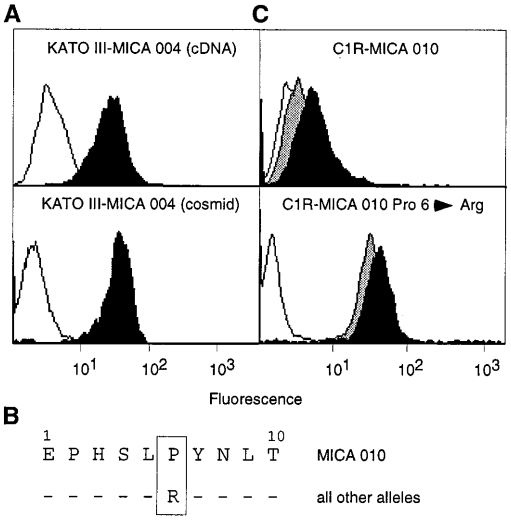

Fig. 2A–C AGS and KATO III cells express a single defective allele of *MICA*. **A** KATO III cells were able to express MICA after stable transfection with a cDNA or cosmid (cosmid M17A; Bahram et al. 1994) encoding *MICA004*. Fluorescence profiles show transfected (*filled*) and nontransfected (*open*) cells stained with mAb 2C10. **B** AGS and KATO III MICA mRNA corresponds to a single allele, *MICA010*, which has a proline for arginine substitution at position 6 in the α1 domain. **C** No significant expression of MICA was observed with numerous C1R cells stably transfected with *MICA010* cDNA cloned by reverse transcription-polymerase chain reaction from KATO III cells. Reversal of the proline to arginine substitution and transfection restored expression. Cells were stained with mAb 2C10 (*black profiles*) or mAb 6D4 (*gray profiles*). *Open profiles* are stainings of untransfected cells

Steinle et al. 1998). Expression of cloned *MICA010* cDNA failed to produce significant amounts of surface MICA on numerous isolates of stably transfected C1R cells that were screened by flow cytometry, using mAbs specific for epitopes in the $\alpha1\alpha2$ (mAbs 2C10 and 6D4) or $\alpha3$ domains of MICA (mAb 6D5) (Fig. 2C) (Groh et al. 1996, 1998). However, reversal of the proline substitution to arginine, by exchange of a 5′-end *Sal*I-*Eco*0109I restriction fragment from *MICA004* cDNA, which differs at no other position in the $\alpha1\alpha2$ domains, restored surface expression of MICA on transfected C1R and KATO III cells (Fig. 2C). Thus, the single proline substitution abolished expression of the *MICA010* allele. In the crystal structure of MICA, this substitution blocks a β-sheet hydrogen bond with the histidine carbonyl at position 27 on the $\beta2$ strand (Li et al. 1999). Moreover, this substitution is incompatible with β-sheet secondary structure, thus likely interfering with protein folding.

There are only limited data on the frequencies of *MICA010* in human populations. In a study population of 242 Caucasians, the frequency of *MICA010* was 5% (Petersdorf et al. 1999), and among 114 unrelated Japanese, 12.5% (Komatsu-Wakui et al. 1999). In homozygotes, loss of MICA function may or may not be compensated by MICB, thus leaving open the question of potential immunological consequences in such individuals. However, *MICA010* is not the only loss-of-expression variant identified. The entire *MICA* gene appears to be deleted in Japanese individuals with an *HLA-B*4801* haplotype (Komatsu-Wakui et al. 1999). Interestingly, some of these also have a nonfunctional *MICB* gene due to a premature stop codon (Ando et al. 1997). This *MICA* and *MICB* loss haplotype occurs at a frequency of about 3.8% in Japanese (Komatsu-Wakui et al. 1999). Homozygous individuals are healthy, with no deficiency being immediately apparent. Thus, expression of MICA/B may not be essential for overall immunological fitness but may complement, and be redundant with, other arms of immune defense. However, subtle consequences of loss of *MICA* and/or *MICB* may become observable with knowledge of the physiological significance of these molecules and their interactions with NK and T cells. Information on expression variants of *MICA* and *MICB* may prove useful in that regard.

Acknowledgements We thank A. Steinle for help. This work was supported by National Institute of Health Grant CA 18221.

References

Ando H, Mizuki N, Ota M, Yamzaki M, Ohno S, Goto K, Miyata Y, Wakisaka K, Bahram S, Inoko H (1997) Allelic variants of the human MHC class I chain-related B gene (MICB). Immunogenetics 46:499–508

Bahram S, Bresnahan M, Geraghty DE, Spies T (1994) A second lineage of mammalian major histocompatibility complex class I genes. Proc Natl Acad Sci USA 91:6259–6263

Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T (1999) Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 285:727–729

Fodil N, Laloux L, Wanner V, Pellet P, Hauptmann G, Mizuki N, Inoko H, Spies T, Theodorou I, Bahram S (1996) Allelic repertoire of the human MHC class I MICA gene. Immunogenetics 44:351–357

Groh V, Bahram S, Bauer S, Herman A, Beauchamp M, Spies T (1996) Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. Proc Natl Acad Sci USA 93:12445–12450

Groh V, Steinle A, Bauer S, Spies T (1998) Recognition of stress-induced MHC molecules by intestinal epithelial γd T cells. Science 279:1737–1740

Groh V, Rhinehart R, Secrist H, Bauer S, Grabstein KH, Spies T (1999) Broad tumor-associated expression and recognition by tumor-derived γδ T cells of MICA and MICB. Proc Natl Acad Sci USA 96:6879–6884

Komatsu-Wakui M, Tokunaga K, Ishikawa Y, Kashiwase K, Moriyama S, Tsuchiya N, Ando H, Shiina T, Geraghty DE, Inoko H, Juji T (1999) *MIC-A* polymorphism in Japanese and a *MIC-A- MIC-B* null haplotype. Immunogenetics 49:620–628

Lanier LL (1998) NK cell receptors. Annu Rev Immunol 16:359–393

Li P, Willie ST, Bauer S, Morris DL, Spies T, Strong RK (1999) Crystal structure of the MHC class I homologue MIC-A, a γδ T cell ligand. Immunity 10:577–584

Parham P, Barnstable CJ, Bodmer WF (1979) Use of a monoclonal antibody (W6/32) in structural studies of HLA-A, B, C, antigens. J Immunol 123:342–349

Petersdorf EW, Shuler KB, Longton GM, Spies T, Hansen JA (1999) Population study of allelic diversity in the human MHC class I-related MICA gene. Immunogenetics 49:605–612

Steinle A, Groh V, Spies T (1998) Diversification, expression, and γδ T cell recognition of evolutionarily distant members of the MIC family of major histocompatibility complex class I-related molecules. Proc Natl Acad Sci USA 95:12510–12515