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MxA transcripts with distinct first exons and modulation of gene expression levels by single-nucleotide polymorphisms in human bronchial epithelial cells

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Abstract Myxovirus resistance A (MxA) is a major interferon (IFN)-inducible antiviral protein. Promoter single-nucleotide polymorphisms (SNPs) of MxA near the IFN-stimulated response element (ISRE) have been frequently associated with various viral diseases, including emerging respiratory infections. We investigated the expression profile of MxA transcripts with distinct first exons in human bronchial epithelial cells. For primary culture, the bronchial epithelium was isolated from lung tissues with different genotypes, and total RNA was subjected to real-time reverse transcription polymerase chain reaction. The previously reported MxA transcript (T1) and a recently registered transcript with a distinct 5′ first exon (T0) were identified. IFN-β and polyinosinic–polycytidylic acid induced approximately 100-fold higher expression of the T1 transcript than that of the T0 transcript, which also had a potential ISRE motif near its transcription start site. Even without inducers, the T1 transcript accounted for approximately two thirds of the total expression of MxA, levels of which were significantly associated with its promoter and exon 1 SNPs (rs17000900, rs2071430, and rs464138). Our results suggest that MxA observed in respiratory viral infections is possibly dominated by the T1 transcript and partly influenced by relevant 5′ SNPs.

Keywords Myxovirus resistance A · Single-nucleotide polymorphism · Human bronchial epithelial cells · Transcript variants

Abbreviations

LD	Linkage disequilibrium
HBE	Human bronchial epithelial
ISRE	Interferon-stimulated response element

Introduction

The interferon (IFN) system plays an important role in innate immunity against pathogens. When viral components are detected by pattern recognition receptors, infected cells produce type I (α and β) and type III (λ) IFNs (Randall and Goodbourn 2008). Binding of IFNs to their specific receptors leads to the induction of more than 300 IFN-stimulated genes, including myxovirus resistance A (MxA), also known as the myxovirus (influenza virus) resistance 1, IFN-inducible protein p78 (mouse) (MX1) gene. Following

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IFN-induced expression, MxA is thought to form oligomeric rings around the nucleocapsid structures of viruses, thereby inhibiting their transcriptional and replicative functions (Haller and Kochs 2011).

The promoter of the human MxA transcript in the original report contains two IFN-stimulated response elements (ISRE), ISRE1 and ISRE2, near the transcription start site, and both are involved in IFN responsiveness (Ronni et al. 1998). The IFN-stimulated gene factor 3 complex binds to the most proximal ISRE1 and the second ISRE2. ISRE1 is essential for MxA promoter activation, whereas ISRE2 has an enhancing effect in the presence of activated ISRE1 (Ronni et al. 1998). IFN regulatory factor 3 can only bind to ISRE2 for enhancing promoter activation (Holzinger et al. 2007). Around ISRE2, there are two single-nucleotide polymorphisms (SNPs) at nucleotide positions −88 and −123, which confer differences in the promoter activity and binding affinity to nuclear proteins (Hijikata et al. 2001; Ching et al. 2010). Promoter SNPs of MxA are reportedly associated with diseases, including hepatitis C (Hijikata et al. 2000; Hijikata et al. 2001), hepatitis B (Peng et al. 2007), multiple sclerosis (Furuyama et al. 2006), and subacute sclerosing panencephalitis (Torisu et al. 2004). We previously reported the association of MxA promoter SNP with the severity of severe acute respiratory syndrome (SARS; Hamano et al. 2005), and Ching et al. (2010) reported its association with susceptibility to SARS in a larger case–control study. However, the expression levels of MxA have been analyzed only in peripheral blood mononuclear cells (PBMC) or liver cells (Fernandez-Arcas et al. 2004; Kong et al. 2007; Abe et al. 2011; McGilvray et al. 2012).

Because MxA has a pivotal role in host defense against not only SARS coronavirus but also other respiratory viruses such as influenza virus (Haller and Kochs 2011), it is important to characterize the expression profile of MxA in human bronchial epithelial (HBE) cells, a site for replication of many respiratory viruses. In addition, a new transcript variant with alternative 5′ untranslated exons starting 5.5 kb upstream of the original exon 1 has recently been registered in the public database (NM_001144925.1). In the present study, we analyzed the expression patterns of MxA transcripts with distinct first exons. We further investigated the possible effects of their 5′ SNPs on gene expression levels in a panel of primary cultured HBE cells with different genotypes.

Materials and methods

Cell culture

The study protocol was approved by the ethical committee of the National Center for Global Health and Medicine (formerly, International Medical Center of Japan). Primary cultured HBE cells were obtained from the cancer-free bronchi of surgically resected lungs after obtaining written informed consent from the individuals concerned, all of whom were Japanese. HBE cells (n=38) were isolated and cultured as described previously (Gray et al. 1996) and used after three–five passages in this study. In brief, HBE cells were seeded at a density of 5×10^5/well onto collagen-coated six-well Transwell plates (Corning Inc., Corning, NY, USA) and cultured in bronchial epithelial growth medium (Bio-Whittaker, Walkersville, MD, USA) for 24 h. Thereafter, HBE cells (n=3) were stimulated with 1,000 IU/ml IFN-α (PeproTech EC Ltd., London W6 8LL, UK), 1,000 IU/ml IFN-β (Biosource International, Camarillo, CA, USA), 100 μg/ml polyinosinic–polycytidylic acid [poly(I:C); Sigma-Aldrich, St. Louis, MO, USA], 10 ng/ml IFN-γ (R&D Systems, Minneapolis, MN, USA), 50 ng/ml TNF-α (R&D Systems), 20 μg/ml lipopolysaccharide (LPS; Sigma-Aldrich), 10 μg/ml α-defensin 1 (Peptide Institute Inc., Osaka, Japan), 10 μg/ml β-defensin 1 (Peptide Institute Inc.), and 10 μg/ml β-defensin 2 (Peptide Institute Inc.) for 24 h and then harvested. Unstimulated HBE cells (n=38) and those stimulated with 100 μg/ml poly(I:C) for 24 h (n=29) or with 1,000 IU/ml IFN-β for 12 h (n=9) were harvested, and gene expression levels were then analyzed. To assess time-dependent changes in mRNA expression, BEAS-2B cells (ATCC number CRL-9609) were stimulated with 100 μg/ml poly(I:C) for 6, 12, 24, and 48 h.

Real-time reverse transcription polymerase chain reaction

We designated the MxA transcript originally reported by Horisberger et al. (1990) (NM_002462.3) as the T1 transcript and the new transcript variant in the public database (NM_001144925.1) as the T0 transcript. Distinct exons used in the T0 transcript are shown as exons 0a, 0b, and 0c (Fig. 1). Translational start codons of both the T1 and T0 transcripts originate from exon 5, indicating that exons 0a–0c and exons 1–4 are all 5′ untranslated exons.

Total RNA of the cells was extracted using the RNeasy Mini Kit (Qiagen, Hamburg, Germany). Human Total RNA Master panel II (Clontech, Mountain View, CA, USA) was used to investigate gene expression in various tissue types. Most of the tissue RNA in this panel consisted of pooled RNA from two or more donors, and their genotypes were not available. One microgram of total RNA was subjected to RT with random nonamers using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). MxA mRNA expression was analyzed by real-time reverse transcription polymerase chain reaction (RT-PCR) using SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) and CFX96 (BioRad, Hercules, CA, USA). Sense and antisense primers were located in exons 0b and 0c (5′-CCAGAGCAACTCCACACCGGGTGC-3′ and 5′-GCTATGGTTCCAATCCAGGTGATC-3′) for the T0 transcript and exons 1 and 2

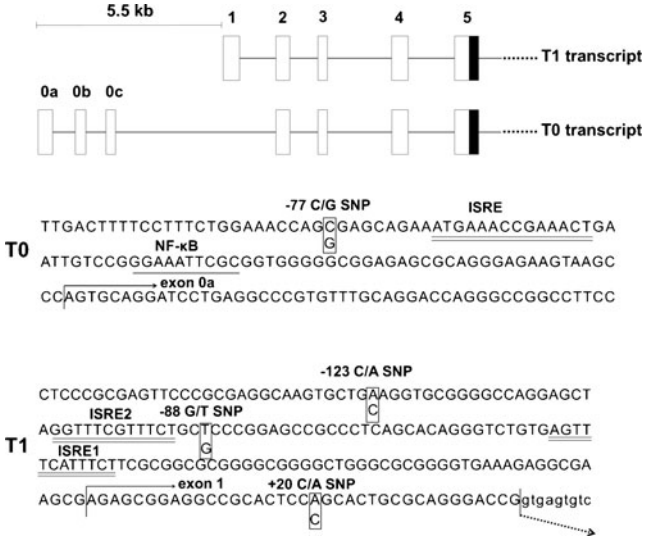

Fig. 1 Alternate splicing of 5′ exons in MxA. The 5′ genomic structure of MxA and nucleotide sequences around the transcription start sites of the T0 and T1 transcripts are shown. *White boxes* represent the untranslated mRNA sequence, and *black boxes* represent the translated sequence. Potential ISREs are *double underlined*, NF-κB binding site is *underlined*, and promoter and exon 1 SNPs are *boxed*. The transcription start site (Horisberger et al. 1990) and nucleotide positions shown in the T1 transcript are displayed in accordance with MxA promoter analysis by Ronni et al. (1998)

(5′-GCACTGCGCAGGGACCG-3′ and 5′-TGGG-TGAG-CAGGTGGGCGGCA-3′) for the T1 transcript. PCR conditions consisted of 40 cycles of denaturation for 15 s at 95 °C and annealing and extension for 1 min at 60 °C. Specific target amplification was confirmed by a single peak in the dissociation curve. The mRNA copy numbers between different transcripts were compared using the absolute quantification method (Leong et al. 2007). RT-PCR products were purified using the Wizard PCR Preps DNA Purification System (Promega, Fitchburg, WI, USA), and their copy numbers were calculated from the DNA concentration determined by measuring the absorbance at 260 nm. The standard curve was generated with a serial fivefold dilution of each RT-PCR product, and the linear dependence of the threshold cycles was confirmed from the template concentrations. We used the β-actin gene (primers listed in Online Resource 1) to normalize the expression of MxA for calculating the relative amounts of mRNA of each transcript. The TaqMan Gene Expression Assay (Hs00182073_m1) (Applied Biosystems, Foster City, CA, USA) that amplifies exons 16–17 of MxA was used with TaqMan Universal Master Mix II (Applied Biosystems) in the StepOne Plus Real-Time PCR System (Applied Biosystems), and the relative amount of total transcripts, indicating the overall expression of MxA, was calculated using the standard curve method with glyceraldehyde 3-phosphate dehydrogenase as an internal control.

Rapid amplification of 5′ cDNA end

RNA ligase-mediated rapid amplification of 5′ cDNA end (5′ RACE) was performed using total RNA from IFN-β-stimulated HBE cells to determine the transcription start site of T0 using the First-Choice RLM-RACE Kit (Ambion, Austin, TX, USA). Gene-specific primers are listed in Online Resource 1. PCR products were sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) using a 3130xl Genetic Analyzer (Applied Biosystems).

Screening and genotyping of polymorphisms in the 5′ region

Genomic DNA was extracted from HBE cells (n=38) using the QIAamp DNA Mini Kit (Qiagen). The 5′ upstream region of the transcription start site for the T0 transcript was amplified with two overlapping PCR products, and the amplified products were sequenced using appropriate inner primers. Three SNPs, −123 C/A (rs17000900), −88 G/T (rs2071430), and +20 C/A (rs464138), two in the promoter and one in exon 1 of the T1 transcript, were genotyped by PCR and restriction fragment length polymorphism methods (Hamano et al. 2005), with Pst I (Takara Bio) for rs17000900, Hha I (Takara Bio) for rs2071430, and Bpm I (New England Biolabs, Ipswich, MA, USA) for rs464138. The primers are listed in Online Resource 1. Linkage disequilibrium (LD) between promoter SNPs was analyzed using Haploview (v. 4.2) (Barrett et al. 2005).

Statistical analysis

All data were expressed as mean±standard error of the mean (SEM). To assess the relationship between the number of single alleles of −123 C/A, −88 G/T, and +20 C/A SNPs and expression levels of the transcript variants, a simple linear regression model was applied (JMP, version 9.0.0; SAS Institute Inc., Cary, NC, USA). A multiple linear regression model was also applied to assess the combined effects of these SNPs on expression of the T1 transcript. The numbers of alleles of the three abovementioned SNPs were incorporated in the model as explanatory variables. Correlations of the total amount of the MxA transcripts with expression levels of the T0 and T1 transcripts were further analyzed using Spearman's rank correlation coefficient. A p value <0.05 was considered to be statistically significant.

Results

Expression patterns of MxA transcripts in human tissues

The originally reported MxA transcript, T1, and a recently registered transcript variant, T0, were both successfully

amplified by RT-PCR from various human tissues (Fig. 2). Expression of the T1 transcript was predominant in the tissues examined, including the lung and trachea, whereas expression of the T0 transcript was inconspicuous, except in the testis and adrenal gland.

Induction patterns of MxA transcripts in HBE cells incubated with type I IFNs and other stimuli

MxA transcripts, T0 and T1, were both detected in the unstimulated primary cultured HBE cells (n=3), and their expression was markedly induced by type I IFNs and poly(I:C), although induction of the T1 transcript was much stronger than that of the T0 transcript (Fig. 3). IFN-γ, TNF-α, and α-defensin 1 also induced expression of the T1 transcript to a lesser extent, whereas this increase was not observed in the T0 transcript.

Genomic structure and genetic polymorphisms in the 5′ upstream regions of MxA transcripts with distinct first exons

Because the transcript variant T0 with alternatively spliced exons was moderately induced by type I IFNs, 5′ RACE was performed to determine the 5′ end of exon 0a, the transcription start site of T0, in IFN-β-stimulated HBE cells (Fig. 1). A putative ISRE motif and a possible binding site for NF-κB were revealed in the 5′ upstream region of the T0 transcript (Fig. 1). The nearly full-length transcript T0 was amplified with the sense primer in exon 0c and the antisense primer in the last exon 17; however, no alternatively spliced exon was observed in the protein-coding region (data not shown). Although the transcript variant that skips untranslated exons 2 and 4 has also been registered in the public database (NM_001178046.1), its expression level was very low in the HBE cells (data not shown).

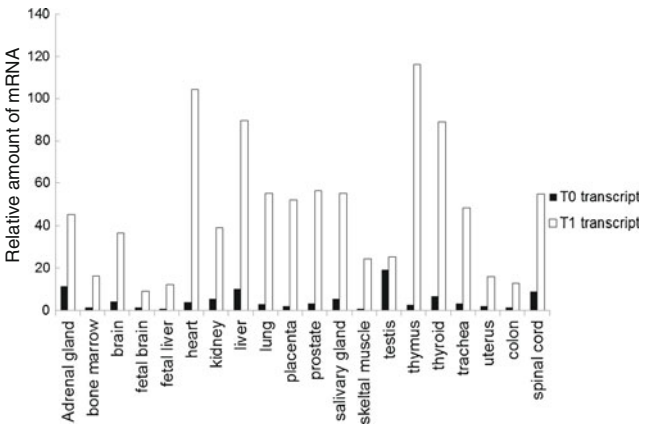

Fig. 2 Relative expression levels of two MxA transcript variants in human tissues. Relative expression levels of the T0 and T1 transcripts in various human tissues were obtained by real-time RT-PCR using a commercial RNA panel, Human Total RNA Master panel II (Clontech). The RNA consisted of pooled RNA from two or more donors. Their genotypes were not available but presumably mixture of different genotypes

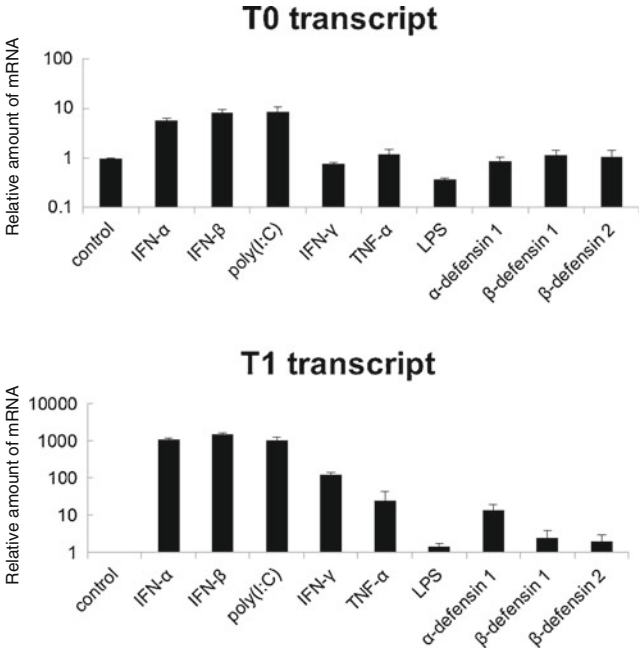

Fig. 3 Induction of T0 and T1 transcript variants by various stimuli in HBE cells. HBE cells (n=3) were stimulated with IFN-α, IFN-β, poly (I:C), IFN-γ, TNF-α, LPS, α-defensin 1, β-defensin 1, and β-defensin 2 for 24 h and then harvested. Expression levels of the T0 and T1 transcripts were compared with those of unstimulated cells by real-time RT-PCR. Fold inductions are shown as the mean±SEM. The genotypes of the promoter SNPs were as follows: sample #1, −77 SNP (rs457274) C/G, −123 SNP (rs17000900) C/A, −88 SNP (rs2071430) G/T, and +20 SNP (rs464138) C/A; sample #2, −77 C/C, −123 C/A, −88 T/T, and +20 A/A and; sample #3, −77 C/G, −123 C/C, −88 G/G, and +20 C/A

Sequence analysis of the 5′ upstream region of the T0 transcript using our DNA samples identified genomic variations, −77 C/G SNP (rs457274) near the putative ISRE motif (Fig. 1), −326 deletion/insertion polymorphism (rs60467231), and −504 A/G SNP (rs12483338). Three other SNPs, −123 C/A (rs17000900), −88 G/T (rs2071430), and +20 C/A (rs464138), near the 5′ end of the T1 transcript were also detected (Fig. 1). As shown in Online Resource 2, −77 C/G SNP of the T0 transcript and the three SNPs near the 5′ end of the T1 transcript were all in strong LD with each other (D'> 0.8, r^2>0.4).

Differences in expression levels of MxA among SNP genotypes

Next, the mRNA expression levels of the T0 and T1 transcripts were analyzed in HBE cells with different genotypes. The expression of the T1 transcript assessed by real-time RT-PCR was 2.3-fold higher than that of the T0 transcript under the unstimulated condition (n=38). Baseline expression of the T1 transcript was significantly higher in proportion to the

number of A alleles of −123 C/A SNP, T alleles of −88 G/T SNP, and A alleles of +20 C/A SNP carried by HBE cells according to a simple regression analysis (p=0.013, p=0.0035, and p<0.0001, respectively) (Fig. 4b). Multiple regression analysis showed that the association of +20 C/A SNP remained significant (p=0.016), whereas the association of −123 C/A and −88 G/T SNPs was insignificant (p=0.67 and p=0.78, respectively). In contrast, baseline expression of the T0 transcript was slightly higher in proportion to the number of C alleles of −77 C/G SNP; however, this increase was not statistically significant (p=0.086) (Fig. 4a).

When the relative expression of the total MxA transcripts was compared with expression of the T1 and T0 transcripts under the unstimulated condition, the overall expression of MxA strongly correlated with expression of the T1 transcript (Spearman's rank correlation coefficient; rs=0.759), whereas it weakly correlated with expression of the T0 transcript (rs=0.388). The total expression of MxA was significantly higher in proportion to the number of −123 A, −88 T, and +20 A alleles (p=0.0004, p<0.0001, and p<0.0001, respectively), which was similar to the linear relationship between the T1 transcript and number of alleles shown above.

When immortalized HBE cell line BEAS-2B was stimulated with poly(I:C), time-course analysis revealed that expression of the T0 transcript was the highest after 24 h, whereas that of the T1 transcript was the highest after 6–12 h of incubation (Online Resource 3). We therefore investigated the expression of T0 and T1 transcripts in HBE cells (n=29) stimulated with poly(I:C) for 24 h. The expression of the T0 transcript increased eightfold, whereas that of the T1 transcript increased 870-fold. Poly(I:C)-induced expression of both transcripts was not associated with either allele of the four SNPs (Fig. 5a, b). When HBE cells (n=9) were stimulated with IFN-β for 12 h, the T0 transcript was induced eightfold, and the T1 transcript was induced 640-fold. IFN-β-induced expression of the transcripts did not vary among genotypes (data not shown).

Discussion

In this study, we investigated the expression profile of MxA by analyzing expression of the original transcript T1 and the transcript variant T0 in primary cultured HBE cells. According to our absolute quantification method using real-time RT-PCR, the amount of the T0 transcript was approximately half of that of the T1 transcript at the baseline level. Although expression of the T0 transcript was also induced by type I IFNs and poly(I:C) and its 5′ proximal region has a potential ISRE motif, IFN-β and poly(I:C) inducibility of the T1 transcript was at least 100-fold higher than that of the

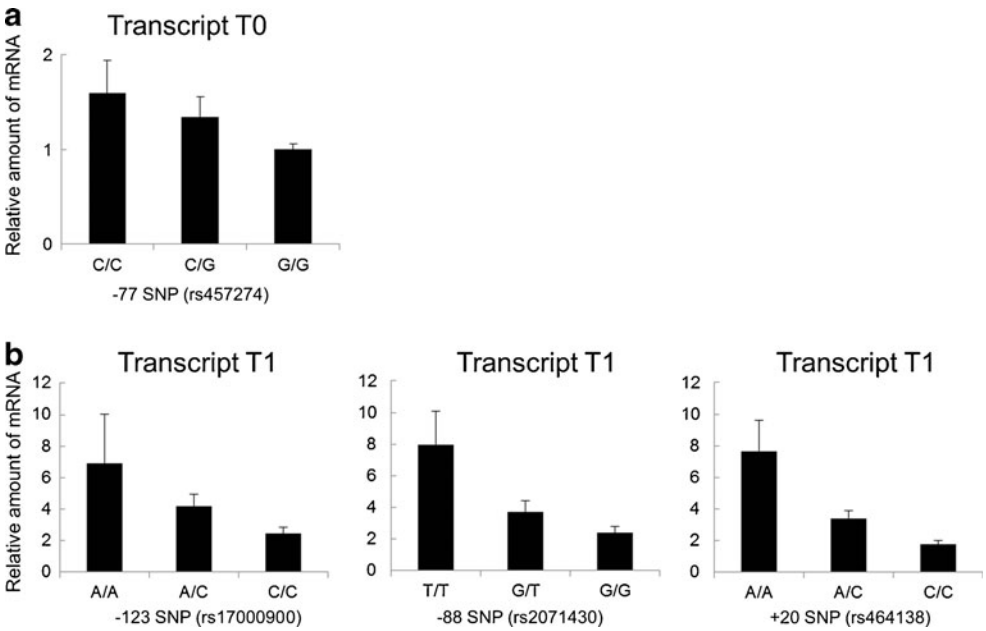

Fig. 4 Differences in the baseline expression of transcript variants among the genotypes of the promoter and exon 1 SNPs in HBE cells. Expression of **a** T0 and **b** T1 transcripts under the unstimulated condition in HBE cells with each genotype of −77 C/G SNP for the T0 transcript (C/C, n=8; C/G, n=18; G/G, n=12), of −123 C/A SNP (A/A, n=2; A/C, n=18; C/C, n=18), of −88 G/T SNP (T/T, n=3; G/T, n=19; G/G, n=16), and of +20 C/A SNP (A/A, n=5; A/C, n=22; C/C, n=11) for the T1 transcript is shown. The relative amounts of mRNA of each transcript compared with that of the T0 transcript in GG cells without poly(I:C) stimulation are shown as mean±SEM. Possible associations between the number of alleles and the amount of the corresponding transcripts were assessed by a simple regression model respectively (p=0.086 for the number of −77 C alleles, p=0.013 for −123 A alleles, p=0.0035 for −88 T alleles, and p<0.0001 for +20 A alleles)

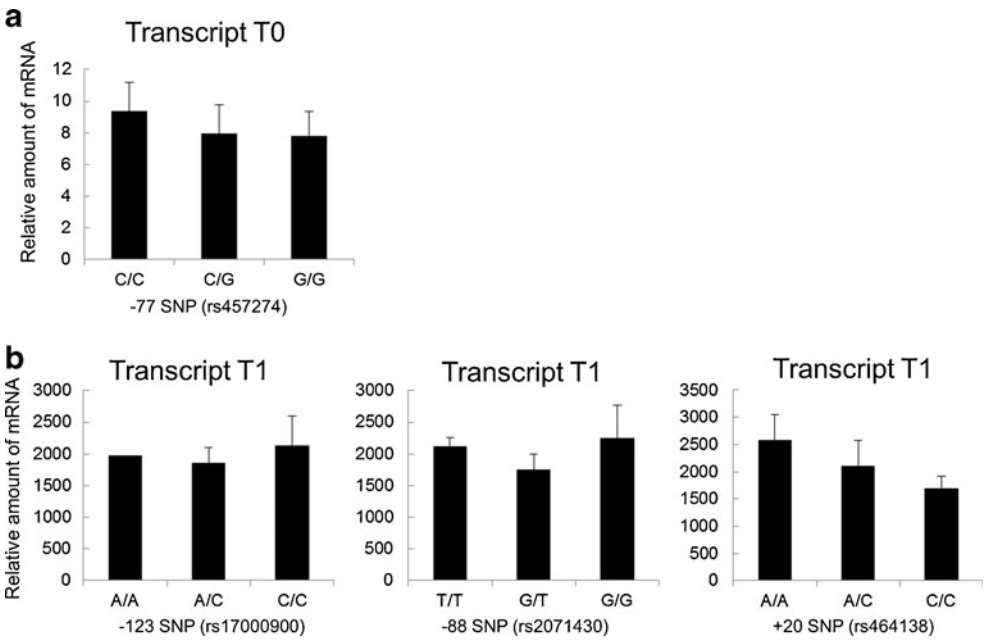

Fig. 5 Differences in the poly(I:C)-induced expression of transcript variants among the genotypes of the promoter and exon 1 SNPs in HBE cells. Expression of **a** T0 and **b** T1 transcripts in the presence of poly(I:C) in HBE cells with each genotype of −77 C/G SNP for the T0 transcript (C/C, $n=5$; C/G, $n=13$; G/G, $n=11$), of −123 C/A SNP (A/A, $n=1$; A/C, $n=12$; C/C, $n=16$), of −88 G/T SNP (T/T, $n=2$; G/T, $n=13$; G/G, $n=14$), and of +20 C/A SNP (A/A, $n=3$; A/C, $n=16$; C/C, $n=10$) for the T1 transcript is shown. The relative amounts of mRNA of each transcript compared with that of the T0 transcript in GG cells without poly(I:C) stimulation are shown as mean±SEM. Possible associations were assessed by a simple regression model ($p=0.647$ for the number of −77 C alleles, $p=0.662$ for −123 A alleles, $p=0.539$ for −88 T alleles, and $p=0.331$ for +20 A alleles)

T0 transcript. This observation implies that MxA response to viral infection of respiratory cells is mostly controlled by the expression of the T1 transcript.

Remarkably increased levels of the T1 transcript after stimulation with type I IFNs or an IFN-inducing agent, poly(I:C), were consistent with the general consensus that the induction of MxA requires type I or type III IFN signaling (Haller and Kochs 2011). However, levels of the T1 transcript were also elevated in HBE cells by other physiological stimuli. Moderate elevation of levels of the T1 transcript in the presence of IFN-γ and TNF-α may be mediated by the secondary induction of type I or type III IFNs. Although Mahanonda et al. (2012) demonstrated the induction of MxA by α-defensin in primary human gingival epithelial cells, the mechanism by which the T1 transcript was upregulated by α-defensin remains unknown. These observations support the idea that the T1 transcript plays a major role in airway diseases.

We also analyzed the expression of T0 transcript with alternative 5′ untranslated exons. Alternative promoter usage is now recognized as a common mechanism in the transcriptional regulation of mammalian genes (Davuluri et al. 2008). Among IFN-stimulated genes, ADAR1 was shown to have alternative promoters (George and Samuel 1999): one promoter contributes to constitutive expression and the other to inducible expression. To our knowledge, no IFN-stimulated genes other than MxA, whose antiviral function is well known (Sadler and Williams 2008), have been reported to possess two IFN-inducible transcripts with distinct first exons. The molecular mechanism for tissue specificity of these transcripts is unknown; however, of note, the promoter of the T0 transcript contains the putative steroidogenic factor-1 binding site (position −636 to −644) thought to be important to testis- and adrenal gland-specific gene expression (Schimmer and White 2010). A study of the expression profiles of MxA in various organs would be interesting.

Although IFN-mediated upregulation of the T0 transcript was moderate in contrast to that of the T1 transcript, baseline levels of the T0 transcript were not negligible in HBE cells. It is thus conceivable that the T0 transcript plays a minor but independent role in the human airway. Furthermore, considering the difference between the time course of mRNA expression of the T0 and T1 transcripts after poly(I:C) stimulation, it is likely that other factors further modulate their induction levels. Because some reports (Aebi et al. 1989; Goetschy et al. 1989; Prescott et al. 2005) indicate the presence of IFN-independent induction of MxA in contrast to the results of Holzinger et al. (2007), it would be worth investigating whether the T0 transcript can be induced through an IFN-independent signaling system in viral infection.

We observed the regulatory effects of −123, −88, and +20 SNPs on mRNA levels of the T1 transcript in HBE cells

under the unstimulated condition. When we evaluated the overall expression of the MxA transcripts by real-time RT-PCR, it was found to be closely correlated with levels of the T1 transcript, suggesting that individual variation of the total expression of MxA is mainly explained by the T1 transcript. Indeed overall levels of MxA as well as expression of the T1 transcript were strongly associated with these three SNPs at baseline levels. It has been repeatedly reported that the minor A allele of −123 SNP and the minor T allele of −88 SNP, which are in strong LD, were associated with the overall transcriptional activity of the gene (Hijikata et al. 2001; Torisu et al. 2004). Expression of MxA was associated with −88 G/T SNP when PBMC cells were stimulated with IFN-α2 for 12 h (Fernandez-Arcas et al. 2004). In one study (Furuyama et al. 2006), the results of a luciferase reporter assay suggested that −123 SNP contributed to basal expression levels of MxA, whereas −88 SNP contributed to the induction of expression by IFNs. Ching et al. (2010) further showed that the −123A allele had a stronger binding affinity to nuclear proteins from unstimulated cells and that the −88 T allele preferentially bound to the protein after IFN-β stimulation. In our study using HBE cells, −123, −88, and +20 SNPs were all associated with baseline expression of the T1 transcript, and according to a multiple regression analysis, among the three SNPs, +20 C/A SNP was still associated with baseline expression of the T1 transcript. This finding may be attributed to the difference in cell type; however, extensive investigation is required to determine the possible effect of +20 SNP or other unknown functional polymorphisms in strong LD. Recently, Tran Thi Duc et al. (2012) reported that three SNPs (−309 C/G, −101 G/A, and +20 C/A) also contributed to the promoter activity in combination with well-known effects of −123 and −88 SNPs. We could not examine −309 and −101 SNPs in our samples because −309 C/G and −101 G/A SNPs were detected only in the African population and their minor allele frequencies were relatively low (Duc et al. 2012).

Under the poly(I:C)-stimulated condition, the +20 SNP also tended to be associated with expression of the T1 transcript in our study; however, this tendency was not statistically significant. When HBE cells were stimulated with IFN-β for 12 h, the same three SNPs were not associated with the expression level. These findings may conflict with the in vitro effects of these 5′ SNPs on the IFN-inducible promoter activity previously assessed by a luciferase reporter system (Hijikata et al. 2001; Torisu et al. 2004; Tran Thi Duc et al. 2012). In our study, however, the mRNA induced by poly (I:C), the dsRNA analog to mimic viral infection, was directly assessed in primary cultured HBE cells, which implies that individual variance of relevant factors such as toll-like receptor 3 and subsequent IFN signaling pathways might have affected the mRNA levels in the IFN-stimulated condition and have masked the independent effects of these promoter SNPs of the MxA gene.

We previously reported that the promoter −88 SNP was associated with severity of SARS in the Vietnamese population (Hamano et al. 2005), and the promoter −123 SNP was associated with SARS in the Chinese population (Ching et al. 2010). According to Chen and Subbarao (2007), IFN induction is completely suppressed in SARS coronavirus-infected cells. Our ex vivo findings that these regulatory SNPs were mainly involved in baseline expression of the T1 transcript support the results of these disease association studies. However, we could not show significant difference in the regulatory effects between −88 and −123 SNPs, possibly because of strong LD between these two SNPs in the Japanese population (r^2=0.83) compared with moderate LD in the Chinese population (r^2=0.39) (Ching et al. 2010).

In conclusion, we characterized the expression profile of the previously known transcript and the transcript variant of MxA and demonstrated a significant effect of its 5′ SNPs on basal expression of the overall transcripts in HBE cells. Our findings may lead to an improved understanding of the association of MxA SNPs with susceptibility to respiratory viral infections.

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Conflict of interest All authors have no conflict of interest on this work.

References

Abe H, Hayes CN, Ochi H, Maekawa T, Tsuge M, Miki D, Mitsui F, Hiraga N, Imamura M, Takahashi S, Kubo M, Nakamura Y, Chayama K (2011) IL28 variation affects expression of interferon stimulated genes and peg-interferon and ribavirin therapy. J Hepatol 54:1094–1101

Aebi M, Fah J, Hurt N, Samuel CE, Thomis D, Bazzigher L, Pavlovic J, Haller O, Staeheli P (1989) cDNA structures and regulation of two interferon-induced human Mx proteins. Mol Cell Biol 9:5062–5072

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265

Chen J, Subbarao K (2007) The immunobiology of SARS. Annu Rev Immunol 25:443–472

Ching JC, Chan KY, Lee EH, Xu MS, Ting CK, So TM, Sham PC, Leung GM, Peiris JS, Khoo US (2010) Significance of the myxovirus resistance A (MxA) gene −123C>a single-nucleotide polymorphism in suppressed interferon beta induction of severe acute respiratory syndrome coronavirus infection. J Infect Dis 201:1899–1908

Davuluri RV, Suzuki Y, Sugano S, Plass C, Huang TH (2008) The functional consequences of alternative promoter use in mammalian genomes. Trends Genet 24:167–177

Duc TT, Farnir F, Michaux C, Desmecht D, Cornet A (2012) Detection of new biallelic polymorphisms in the human MxA gene. Mol Biol Rep 39:8533–8538

Fernandez-Arcas N, Blanco A, Gaitan MJ, Nyqvist M, Alonso A, Reyes-Engel A (2004) Differential transcriptional expresion of

the polymorphic myxovirus resistance protein A in response to interferon-alpha treatment. Pharmacogenetics 14:189–193

Furuyama H, Chiba S, Okabayashi T, Yokota S, Nonaka M, Imai T, Fujii N, Matsumoto H (2006) Single nucleotide polymorphisms and functional analysis of MxA promoter region in multiple sclerosis. J Neurol Sci 249:153–157

George CX, Samuel CE (1999) Human RNA-specific adenosine deaminase ADAR1 transcripts possess alternative exon 1 structures that initiate from different promoters, one constitutively active and the other interferon inducible. Proc Natl Acad Sci U S A 96:4621–4626

Goetschy JF, Zeller H, Content J, Horisberger MA (1989) Regulation of the interferon-inducible IFI-78K gene, the human equivalent of the murine Mx gene, by interferons, double-stranded RNA, certain cytokines, and viruses. J Virol 63:2616–2622

Gray TE, Guzman K, Davis CW, Abdullah LH, Nettesheim P (1996) Mucociliary differentiation of serially passaged normal human tracheobronchial epithelial cells. Am J Respir Cell Mol Biol 14:104–112

Haller O, Kochs G (2011) Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity. J Interferon Cytokine Res 31:79–87

Hamano E, Hijikata M, Itoyama S, Quy T, Phi NC, Long HT, Ha LD, Ban VV, Matsushita I, Yanai H, Kirikae F, Kirikae T, Kuratsuji T, Sasazuki T, Keicho N (2005) Polymorphisms of interferon-inducible genes OAS-1 and MxA associated with SARS in the Vietnamese population. Biochem Biophys Res Commun 329:1234–1239

Hijikata M, Mishiro S, Miyamoto C, Furuichi Y, Hashimoto M, Ohta Y (2001) Genetic polymorphism of the MxA gene promoter and interferon responsiveness of hepatitis C patients: revisited by analyzing two SNP sites (−123 and −88) in vivo and in vitro. Intervirology 44:379–382

Hijikata M, Ohta Y, Mishiro S (2000) Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt −88) correlated with the response of hepatitis C patients to interferon. Intervirology 43:124–127

Holzinger D, Jorns C, Stertz S, Boisson-Dupuis S, Thimme R, Weidmann M, Casanova JL, Haller O, Kochs G (2007) Induction of MxA gene expression by influenza A virus requires type I or type III interferon signaling. J Virol 81:7776–7785

Horisberger MA, McMaster GK, Zeller H, Wathelet MG, Dellis J, Content J (1990) Cloning and sequence analyses of cDNAs for interferon- and virus-induced human Mx proteins reveal that they contain putative guanine nucleotide-binding sites: functional study of the corresponding gene promoter. J Virol 64:1171–1181

Kong XF, Zhang XX, Gong QM, Gao J, Zhang SY, Wang L, Xu J, Han Y, Jin GD, Jiang JH, Zhang DH, Lu ZM (2007) MxA induction may predict sustained virologic responses of chronic hepatitis B patients with IFN-alpha treatment. J Interferon Cytokine Res 27:809–818

Leong DT, Gupta A, Bai HF, Wan G, Yoong LF, Too HP, Chew FT, Hutmacher DW (2007) Absolute quantification of gene expression in biomaterials research using real-time PCR. Biomaterials 28:203–210

Mahanonda R, Sa-Ard-Iam N, Rerkyen P, Thitithanyanont A, Subbalekha K, Pichyangkul S (2012) MxA expression induced by alpha-defensin in healthy human periodontal tissue. Eur J Immunol 42:946–956

McGilvray I, Feld JJ, Chen L, Pattullo V, Guindi M, Fischer S, Borozan I, Xie G, Selzner N, Heathcote EJ, Siminovitch K (2012) Hepatic cell-type specific gene expression better predicts HCV treatment outcome than IL28B genotype. Gastroenterology 142:1122–1131

Peng XM, Lei RX, Gu L, Ma HH, Xie QF, Gao ZL (2007) Influences of MxA gene−88 G/T and IFN-gamma +874 A/T on the natural history of hepatitis B virus infection in an endemic area. Int J Immunogenet 34:341–346

Prescott J, Ye C, Sen G, Hjelle B (2005) Induction of innate immune response genes by Sin Nombre hantavirus does not require viral replication. J Virol 79:15007–15015

Randall RE, Goodbourn S (2008) Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. J Gen Virol 89:1–47

Ronni T, Matikainen S, Lehtonen A, Palvimo J, Dellis J, Van Eylen F, Goetschy JF, Horisberger M, Content J, Julkunen I (1998) The proximal interferon-stimulated response elements are essential for interferon responsiveness: a promoter analysis of the antiviral MxA gene. J Interferon Cytokine Res 18:773–781

Sadler AJ, Williams BR (2008) Interferon-inducible antiviral effectors. Nat Rev Immunol 8:559–568

Schimmer BP, White PC (2010) Minireview: steroidogenic factor 1: its roles in differentiation, development, and disease. Mol Endocrinol 24:1322–1337

Torisu H, Kusuhara K, Kira R, Bassuny WM, Sakai Y, Sanefuji M, Takemoto M, Hara T (2004) Functional MxA promoter polymorphism associated with subacute sclerosing panencephalitis. Neurology 62:457–460

Tran Thi Duc T, Desmecht D, Cornet A (2012) Functional characterization of new allelic polymorphisms identified in the promoter region of the human MxA gene. Int J Immunogenet. doi:10.1111/j.1744-313X.2012.01153.x