南臺灣族群 MxA 基因起動子之核苷酸序列分析之研究

王惠亮¹、巴子晨²

摘 要

人類 MxA 蛋白質是干擾素誘導的蛋白質之一，具有對抗 influenz 病毒及其它 RNA 病毒的抗病毒性，故被認為在寄主對抗某些特定的病原中，扮演重要的角色。MxA 蛋白質已被確認具結構與功能和鼠科 Mx1 蛋白質類似。一個共同的特性，是在某些器官中，MxA 蛋白質被干擾素-α 或干擾素-β 刺激反應而合成。基因表現的實驗已顯示，MxA 基因起動子中，至少有一區域含有對干擾素有反應的類似干擾素刺激反應序列(interferon stimulated response element like sequence；ISRE-like sequence)。本實驗隨機選取了 30 位南台灣閩南人，進行 MxA 基因起動子核苷酸序列分析。實驗結果顯示，MxA 基因起動子無 TATA 盒子(TATA-box)或 CAAT 盒子(CAAT-box)，但有一富含 GC 核苷酸之區域。南台灣閩南族群之 MxA 基因起動子核苷酸序列與 NCBI 基因庫(NCBI GenBank)中 MxA 基因起動子之核苷酸序列相同度為 98%。其中，在-2 nt (-2 位置之核苷酸) 與 -3 nt 間有一插入 Guanlyc acid(G)核苷酸，此位置恰位於一般所調的起始子(initiator)區域內。在-32 nt 與-33 nt 之間插入的 Guanlyc acid(G)核苷酸，恰位於所推測的 SPI 結合位置內。而-41 nt 位置缺乏之 Cytidylic acid(C)核苷酸，恰位於 ISRE1 之下游一個核苷酸位置。在起動子-89 nt 及-124 nt 位置上，則發現分為 G/T 與 A/C 的對偶核苷酸多型性。此兩個核苷酸位置使用限制片斷長度多型性分析(restriction fragment length polymorphism assay；RFLP assay)，確認所屬的合子(zygote)種類。結果顯示 C/C 同型合子與 G/G 同型合子，彼此間顯示高度的關連性(P<0.05)。此外，兩個 SNP 間亦顯示高度的關連性，所有 30 個個體中，其中在 -124 nt 位置上具有 Cytidylic acid (C)核苷酸有 29 個個體。而在 29 個個體中，有 28 個個體在同一位置具有 Cytidylic acid (C)核苷酸，和在-89 nt 位置上具有 Guanlyc acid (G)核苷酸，其關連性達 96.6% (28/29)。而在-89 nt 位置上具有 Thymidylic acid (T)核苷酸的 11 個個體中，有 9 個個體本身在 -124 nt 位置上亦有 Adenylic acid (A)核苷酸，其關連性達 81.8%(9/11)。

關鍵字：MxA 基因、起動子、單核苷酸多型性、類似干擾素刺激反應序列、干擾素

投稿日期：民國 100 年 9 月 20 日；接受刊登日期：民國 100 年 12 月 16 日

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Analysis of MxA gene Promoter from Southern Min Group of Southern Taiwanese

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Abstract

The human MxA protein is an interferon-inducible protein that confers resistance to influenza virus and other RNA viruses. MxA proteins are synthesized in response to stimulation by type I (αβ) interferon in specific organ. Gene expression suggested that at least a region of nucleotide sequence in the MxA gene promoter contains ISRE (interferon stimulated response element). The nucleotide sequences of MxA gene promoters of 30 randomly selected individuals of Southern Min group in southern Taiwan were analyzed. There was no apparent TATA box or CAAT box but a GC-rich region in the MxA gene promoter. Identity of nucleotide sequence of MxA gene promoter from Southern Min group of southern Taiwanese was 98% in comparison with that of NCBI (National Center for Biotechnology Information) GenBank data library. The result demonstrated that a Guanylic acid and a Cytidylic acid were inserted between nucleotide positions -2 and -3 as well as -32 and -33 of the initiator region and the putative SP1 protein binding site of MxA gene promoter, respectively. A deleted Cytidylic acid was found at nucleotide position -41 which was one nucleotide downstream of the ISRE1 region. G/T and A/C allelic single nucleotide polymorphisms (SNPs) were found at nucleotide positions -89 and -124, respectively. A RFLP (restriction fragment length polymorphism) assay was used for identifying the type of nucleotide or zygote of these two sites. The result showed that C/C and G/G homozygotes exhibited high correlation to each other (P< 0.05). Furthermore, among 30 individuals two SNPs showed a high linkage. 29 of 30 individuals had C at nucleotide position -124 and 28 of 30 individuals had C at nucleotide position -124 as well as G at nucleotide position -89, respectively. The linkage reached 96.6% (28/29). 11 of 30 individuals had T at nucleotide position -89 and 9 of 30 individuals had T at nucleotide position -89 as well as A at nucleotide position -124, respectively. The linkage reached 81.8% (9/11).

Keywords: MxA gene, Promoter, single nucleotide polymorphism (SNP), interferon stimulated response element like sequence (ISRE-like sequence), Interferon

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Introduction

Studies of the Mx gene have begun with the finding of a mouse strain resistant to influenza virus infection [1]. Both MxA and MxB are interferon-induced proteins of human cells and are related to the Mx1 protein of murine [2]. The human 76-kDa MxA protein was identified as a structural and functional homologue of the murine Mx1 protein [3, 4]. In contrast to the nuclear murine protein Mx1, both human MxA and MxB accumulate in the cytoplasm of IFN-treated cells [2]. It has been shown that the gene encoding the murine 72-kDa Mx1 is located on chromosome 16 [1]. Human MxA gene is located on chromosome 21 [5]. The length of mRNA of MxA gene is 2787 bp (NCBI GenBank: http://www.ncbi.nih.gov/Genbank/: accession: M33882; complete cds, 2002).

The Mx proteins are present in most vertebrates but the anti-virus spectra are different. For example, the murine Mx1 protein inhibits proliferation of influenza virus but not vesicular stomatitis virus (VSV), while the human MxA protein is effective for both viruses [1]. The human MxB protein is structurally similar to the MxA protein, although the anti-influenza virus property is much lower than that of MxA [1].

In human peripheral blood mononuclear cells (PBMCs), macrophages are the principal producers of MxA protein [6]. A common trait is that Mx proteins are synthesized in response to stimulation by IFN-α or β but not by IFN-γ in a limited number of organs, i.e. liver cells, peripheral blood cells and nerve cells [1].

The mechanism of IFN-dependent induction of Mx proteins remains unclear, although the three motifs similar to that of ISRE (interferon stimulated response element) have been found in its transcription regulatory regions [1]. An IFN-inducible expression of the luciferase gene assay driven by MxA gene promoter demonstrated that the region between nucleotide positions -59 and -30 upstream of the transcription initiation site contains an IFN-responsive element [1]. Moreover, the basal promoter activity is drastically reduced when the region between nucleotide positions -165 and -117 is deleted. This may be due to the presence of a regulatory sequence(s) in this region involved in activation of transcription [1].

There are several key points at which gene expression is controlled in eukaryotic cells, including activation of gene structure, transcription initiation, termination of elongation, nuclear RNA processing, mRNA transport, mRNA translation, and mRNA stability [7]. Promoter is a region of DNA involved in binding of RNA polymerase to initiate transcription [8]. Promoter can function not only to bind RNA polymerase, but also to specify the places and times that transcription can occur from that gene [9]. The core promoter is generally defined as the minimal set of DNA sequence (typically about 40 base pairs) that is sufficient to direct the accurate initiation of transcription by RNA polymerase II (PNAP II) and the basal factors. There are several known core promoter motifs, which include the TATA box, the TFIIB recognition element (BRE), the initiator (Inr), and the downstream promoter element (DPE) [8].
Now, the renewed and extensive interest in genome polymorphism signifies a development in human genetics research that will have a major impact upon population genetics, drug development, forensics, cancer and genetic disease research [10]. In order to investigate the variation of MxA gene promoter, which may influence the expression of MxA gene, we analyzed the structure of MxA gene promoter from Southern Min group of southern Taiwanese.

**Materials and Methods**

**Amplification of the promoter of MxA gene**

30 randomly selected human individuals of Southern Min group were obtained from Kaohsiung Medical University in Southern Taiwan. The genomic DNA was extracted from peripheral blood by using a DNA Blood Kit (QIAGEN, Germany) and quantitated by measurement of the optical density at 260 nm and stored at -70°C. In order to amplify the promoter of MxA gene, polymerase chain reactions (PCRs) were performed using the genomic DNA as its template and two fragments of nucleotide sequence of MxA gene as its primers. Two primers for amplification were MxA F01 (5’-ACACACCCGTTCACCCTGGAGAGGCCAG-3’ forward, -569 nt ~ -540 nt) and MxA R02 (5’-TGCGCAGTGCCTGGAGTCCTCTCGCAGCT-3’ reverse, +30 nt ~ +1nt) [11]. The PCRs were carried out in a total volume of 25 µl containing 50 ng/µl genomic DNA, 0.5 µM each primer, 0.06 U/µl Tag DNA polymerase (Amersham Pharmacia Biotech, USA), 50 µM dNTP, and 1 × PCR buffer. PCRs amplifications were performed in a Thermal cycler GeneAmp PCR system 2400 (Perkin Elmer, USA). The cycling conditions for PCRs were set as follows: one cycle at 95°C for 10 minutes (pre-denaturation); 35 cycles at 95°C for 45 seconds (denaturation), 63°C for 45 seconds (annealing), 72°C for 45 seconds (elongation); one cycle 72°C for 7 minutes (final-elongation).

**Analysis of PCRs products of MxA gene promoter**

Electrophoretic analysis of PCRs products of MxA gene promoter were performed in agarose gels under the system of 100 volt and 0.5 × TAE buffer for 30 minutes. Each sample of electrophoretic analysis contained 5 µl PCRs products and 1 µl 6 × loading dye. After the PCR products were dyed using ethidium bromide and then were photographed under the UV light, the size of PCR products of MxA gene promoter was estimated in contrast to DNA marker (100 ~ 1500 bp; Promega, USA).

**Sequencing of MxA gene promoter**

In order to sequence the nucleotide sequence of MxA gene promoter, PCR products were purified by using ExcelBand™ PCR Clean-up Kit (PREMIER, Canada). Department of Biological Science of National Taiwan Normal University was entrusted to sequence the nucleotide sequence of MxA gene promoter by using DYEnamic ET Terminator Cycle Sequencing Kit and MegaBace 500 DNA
Sequencer (Amersham Pharmacia Biotech, USA) with the same primers of PCR.

**Analysis of polymorphism and identity of MxA gene promoter**

After sequencing, we analyzed the polymorphism and identity of nucleotide sequence of MxA gene promoter and compared it with NCBI GenBank (http://www.ncbi.nih.gov/Genbank/) using the Hitachi software of the DNASIS for Windows (version 2.1 DNASIS). The type of zygote was identified according to the result of RFLP (restriction fragment length polymorphism) assay by using the adaptive restriction enzymes which were researched from software of DNASIS. Each reaction of RFLP assay contained 1 μl restriction enzyme (Promega, USA), 2 μl buffer of restriction enzyme, 10 μl DEPC water (diethyl pyrocarbonate water) and 7 μl PCR products in a total volume of 20 μl. Each reaction was incubated at 37℃ for 3 hours and then we carried out an electrophoretic analysis which was the same as above system of electrophoresis in 3% agarose gels for 60 minutes and finally the type of zygote of SNPs was confirmed according to the distribution of the fragments.

Group data were analyzed using Fisher exact test. *p < 0.05 was regarded as statistically significant.

**Result**

The length of PCR products of MxA gene promoter was 603 bp, including nucleotides of primers in all samples (fig. 1). Identity of nucleotide sequence of MxA gene promoter from Southern Min group of southern Taiwanese was 98% in comparison with that of NCBI GenBank (http://www.ncbi.nih.gov/Genbank/; accession:X55639) (fig. 2). Their differences in nucleotide sequence were insertion, substitution and deletion of single nucleotide. In contrast to the NCBI GenBank, if we used the position of NCBI GenBank as a standard position, three insertions of single Guanylic acids (G) between nucleotide positions -2 ~ -3, -32 ~ -33, and -408 ~ -409, and two insertions of single Cytidylic acids (C) between nucleotide positions -388 ~ -389 and -412 ~ -413 were observed. Furthermore, single Guanylic acid substitutes for Thymidylic acid (T) at nucleotide position -213 and a deletion of single Cytidylic acid (C) at nucleotide position -41 were also observed. (table 1 and fig. 2).

According to the positions of this study, there were G/T and C/A allelic SNPs at nucleotide positions -89 and -124, respectively (fig. 2). From the results of sequencing, if a single signal of nucleotide sequence appeared at position of SNP, this sample represented a homozygote (fig. 3A and 3C). If a double signal appeared at position of SNP, this sample might represent a heterozygote (fig. 3B and 3D). The results must be confirmed by using RFLP assay, because of possible interferences in sequencing. CfoI and PstI were used as adaptive restriction enzymes for RFLP assay of this study.

In this study, some regions of nucleotide sequence were similar to several regulatory elements, inclusive of three ISRE-like elements (interferon stimulated response elements) : ISRE1 (AGTTTCATTTCCTTCGC), ISRE2 (GGTTTCGTTTCTGC), and ISRE3 (GAAACCGGGAAA) ;
three putative IL-6 REs (CTGGGA; interleukin-6 response elements) ; one putative NF-κB binding site (GGGAGCCTCCG; nuclear factor-kappa B binding site) ; one putative Sp1 binding site (CGGGGCGGGG; Sp1 protein binding site) (fig.4).

There was no TATA box or CAAT box near the start point, but there was a distinct GC rich region at nucleotide positions -16 ~ -45 in the MxA gene promoter. Above-mentioned Sp1 binding site was just located at GC rich region and allelic SNP of nucleotide position -89 was just located at ISRE2 region. Three IL-6 REs were close to each other within the distance of 13 and 15 nucleotides, respectively. Moreover, ISRE1 was near the start point, which was located at nucleotide positions -43 ~ -58 (fig. 4).

To confirm the type of zygote, CfoI(GCG↓C) and PstI(CTGCA↓G) were used for allelic SNP of nucleotide positions –89 and –124, respectively. In the RFLP assay, G/G homozygote had single visual band of 485 bp. G/T heterozygote had two visual bands of 485 bp and 535 bp. T/T homozygote had single visual band of 535 bp (fig. 5). C/C homozygote had two visual bands of 296 bp and 155 bp. C/A heterozygote had three visual bands of 448 bp, 296 bp, and 155 bp. A/A homozygote had two visual bands of 448 bp and 155 bp (fig. 6). There were 18 G/G homozygotes, 11 G/T heterozygotes and 1 T/T homozygote according to the SNP at nucleotide position – 89. 21 C/C homozygotes, 8 C/A heterozygotes and 1 A/A homozygote according to the SNP at nucleotide position – 124 among 30 randomly selected individuals. The results showed that C/C and G/G homozygotes exhibited high correlation to each other (P<0.05)(table 2). Furthermore, among 30 individuals, two SNPs showed a high linkage. 29 of 30 individuals had C at nucleotide position -124 and 28 of 30 individuals had C at nucleotide position -124 as well as G at nucleotide position -89, respectively. The linkage reached 96.6% (28/29). 11 of 30 individuals had T at nucleotide position -89 and 9 of 30 individuals had T at nucleotide position -89 as well as A at nucleotide position -124, respectively. The linkage reached 81.8% (9/11).

**Discussion**

Promoters of genes have similar structures. Promoters usually have a TATA sequence (TATA box) about 30 base pairs upstream from the start point of transcription, as well as other possible promoter elements further upstream [9]. Most promoters also have CAAT box near nucleotide position -75 and GC box near nucleotide position -90, but they do not exist together sometimes [8]. The TATA box usually is surrounded by GC rich sequences, which may be a factor in its function [8]. Because the TATA box is at a fixed distance from the start point, its recognition is important for the "positioning" of RNA polymerase [8].

The identity of nucleotide sequence of MxA gene promoter from Southern Min group of southern Taiwanese was 98% in comparison with that of NCBI GenBank. In this study, MxA gene promoter had no apparent TATA box, but a distinct GC rich region existed at nucleotide positions -16 ~ -45, which was not near the general nucleotide position -90. However, some researchers found that some
GC rich regions were located at nucleotide positions -40 ~ -70 [8]. It is apparent that MxA gene promoter is a TATA-less promoter. There are many genes (mostly those encoding general metabolic proteins and not cell-specific proteins) that use RNA polymerase II, and those promoters lack the TATA sequence [9]. In this case, some other general promoter-binding proteins bind to the promoter region such as SP1 (SP1 special factor) [9]. The SP1 protein first binds to the GC rich promoter element then binds TF II D (transcription factor II D) either directly or through a TAF (TBP-associated factor). The TF II D is able to begin the cascade of factors that will form the transcription initiation complex and bind an RNA polymerase II protein to the promoter region [9]. In this study, a similar sequence of SP1 protein binding site was found in the GC rich region, so MxA gene may have a similar process of transcription initiation using above-mentioned model. Moreover, the SP1 binding site is close to ISRE1 (interferon stimulated response element 1) and the start point of transcription at the distance of 4 and 28 nucleotides, respectively, so it may be possible that there are mutual effects among SP1 special factor, ISGF3 (interferon stimulated gene factor 3), and all the relational proteins that form the pre-initiation complex. It would be interesting to observe whether those mutual effects influence the transcription initiation of MxA gene.

There is no extensive homology of sequence at the start point, but there is a tendency for the first nucleotide of mRNA to be A, flanked on either side by pyrimidines. This region is called the initiator (Inr). The Inr is contained between positions −3 ~ +5 [8]. The nucleotide position +1 of MxA gene was also A.

In comparison with the NCBI GenBank, all 30 samples have an inserted Guanylic acid between nucleotide positions -2 ~ -3, which are located at general initiator region (Table 1). Theoretically, it is more difficult to separate double strand DNA when the transcription occurs, because this inserted G-C binding forms three hydrogen bonds in the initiator region. It is interesting to observe whether this inserted Guanylic acid would make a difference on the gene expressions between Southern Min and foreign groups.

There was also an inserted Cytidylic acid between nucleotide positions -32 and -33, which was just located at putative SP1 binding site and a deleted Cytidylic acid near the ISRE1 at a distance of one nucleotide. SP1 binding site is important for TATA-less promoter and ISRE1 is necessary for interferon-induced response, so whether this variation would affect the function of promoter requires further investigations.

Test had shown that allelic SNP of nucleotide position −89 just appeared in ISRE2 region and influenced the expression of MxA gene in luciferase reporter assay of Hijikata et al. [12]. The allelic SNP of nucleotide position −124 didn’t exist in ISRE region but it had similar result in luciferase reporter assay. The positions of these two allelic SNPs were different between the report of Ronni et al. [13] and this study, since there was no inserted Guanylic acid (G) between the nucleotide positions -2 ~ -3 in the report of Ronni et al., whose positions were −88 and -123. However, in this study, the types of nucleotides of the two SNPs were the same as the report of Ronni et al., and the former is G or T, the latter is C or A. Both the SNPs of nucleotide position −88 and −89 were located at ISRE2
region, which may influence the expression of MxA gene. If the variation of promoter influences gene expression, host’s antiviral function of MxA gene may be influenced. The structure of promoter of this study is different from other human groups, therefore the antiviral effect of the MxA gene may be different. Moreover, variations may exist in exon or intron, and polymorphisms of promoter together with exon or intron may enhance the change of gene function. It may be required for us to observe whether polymorphism of exon or intron exists in the MxA gene, which may lead to the difference of gene expression between different groups.

The result showed that C/C and G/G homozygotes exhibit a high correlation to each other \( (P<0.05) \). This result is the same as the report of Hijikata et al. [12]. It is interesting to investigate the meaning of evolution and biological medicine about this correlation in the future.

**Acknowledgements**

We thank Chung-Ho Memorial Hospital of Kaohsiung Medical University for providing samples, and Dr. Shih-hsiung Liang for helping on statistical methods.

**References**


Fig.1. Electrophoretic analysis of PCR products of promoter of MxA gene from human individuals of Southern Min group in a 1% agarose gel. Lane M: DNA marker (100~1500 bp; Promega, USA) Lane 1, 2, and 3: represented 3 samples randomly selected from human individuals of Southern Min group, respectively.
Fig. 2. Comparison of nucleotide sequences of MxA gene promoter. The upper sequence was from NCBI GenBank and lower sequence was the sequence of this study. G/T and A/C allelic single nucleotide polymorphisms (SNPs) appeared at nucleotide positions -89 and -124 in this experimental sequence, respectively. The double underlines indicated the +1 ~ +30 nucleotides of exon 1.
Fig. 3. Signals display of nucleotide sequence of MXA gene promoter in sequencing. A and C: If a single signal appeared at nucleotide position –89 and –124 of SNP, they represented G/G and C/C homozygotes, respectively. B and D: If a double signal appeared at nucleotide position –89 and –124 of SNP, they represented G/T and C/A heterozygotes, respectively.
Fig. 4. The positions of potential regulatory elements in the nucleotide sequence of MxA gene promoter of this study. Sp1 = Sp1 special factor. ISRE = interferon stimulated response element. NF-κB = nuclear factor-kappa B. IL-6 RE = interleukin-6 response element.
Fig. 5. Electrophoretic analysis of PCR products of MxA gene promoter by using CfoI RFLP assay in a 3% agarose gel.
Lane M: DNA marker (100 bp ~ 1500 bp; Promega, USA)
Lane 1: G/G homozygote (485 bp)
Lane 2: G/T heterozygote (containing 485 bp and 535 bp)
Lane 3: T/T homozygote (535 bp)

Fig. 6. Electrophoretic analysis of PCR products of MxA gene promoter by using PstI RFLP assay in a 3% agarose gel.
Lane M: DNA marker (100 bp ~ 1500 bp; Promega, USA)
Lane 1: C/C homozygote (containing 296 bp and 155 bp)
Lane 2, 3, and 4: C/A heterozygote (containing 448 bp, 296 bp and 155 bp)
Lane 5: A/A homozygote (containing 448 bp and 155 bp)
Table 1. Comparison of nucleotide sequences of MxA gene promoter from 30 randomly selected individuals of Southern Min group in southern Taiwan with that of NCBI GenBank.

<table>
<thead>
<tr>
<th>Nucleotide position of NCBI GeneBank (nt)</th>
<th>Inserted nucleotide</th>
<th>Substituted nucleotide</th>
<th>Deleted nucleotide</th>
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<tr>
<td>-2～-3</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-32～-33</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-388～-389</td>
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<td></td>
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<td>-408～-409</td>
<td>G</td>
<td></td>
<td></td>
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<td>-412～-413</td>
<td>C</td>
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<tr>
<td>-41</td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>-213</td>
<td></td>
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<td>G substitutes for T</td>
</tr>
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</table>

Table 2. Correlation of different zygotes of allelic SNP in MxA gene promoter, exclusive of T/T and A/A homozygotes.

<table>
<thead>
<tr>
<th>Nucleotide polymorphism of MxA gene promoter</th>
<th>C/C zygote</th>
<th>C/A zygote</th>
<th>( p^* )</th>
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<td>G/G zygote</td>
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<td>1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>G/T zygote</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Fisher exact test. *\( p < 0.05 \) was regarded as statistically significant.