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Molecular Analysis of HLA-DR Gene Expression Induced by IFN-γ in Malignant Melanoma Cell Lines

Jung Lim Lee[1], Yeon Hyang Kim[2], Jae Myun Lee[2], Joo Deuk Kim[2], Se Jong Kim[2], and Jeon Han Park[2]

Abstract

Human leukocyte antigen (HLA) class II molecules are polymorphic cell surface glycoproteins that are crucial for the cellular interaction in immune response. The expression of class II molecules is regulated in a tissue-specific and cytokine-inducible manner, and is mainly restricted to the antigen presenting cells. However, some tumor cells also express class II molecules, and in some class-II-negative tumor cells, class II expression is inducible by interferon (IFN)-γ. However, their expression varies, even though the tumor cells originate from the same histological origin; some tumor cells show strong expression, others show weak or no expression. To determine whether this differential expression of class II molecules on tumor cells is transcriptionally regulated, FACS analysis and Northern hybridization were performed using a panel of melanoma cell lines, IGR3, Malme-3M, SK-Mel-24, and SK-Mel-28 to analyze the cell surface expression and mRNA transcription rate of HLA-DR before and after treatment with IFN-γ. FACS analysis showed that before IFN-γ treatment, IGR3 and Malme-3M cells barely expressed HLA-DR. On the contrary, almost all of the SK-Mel-24 cells (>90%) and a relatively high rate (>50%) of SK-Mel-28 cells expressed HLA-DR. After IFN-γ treatment, HLA-DR expression was induced in Malme-3M cells and SK-Mel-28 cells which displayed elevated levels of HLA-DR expression in a time- dependent manner. However, IGR3 cells never responded to IFN-γ. Northern analysis showed that treatment with IFN-γ led to the steady-state mRNA augmentation of the HLA-DR gene in Malme-3M and SK-Mel-28, whereas in IGR3, IFN-γ did not augment the transcriptional rate of the HLA-DR gene. To further clarify this differential modulation, sequencing analysis of PCR product of the HLA-DR proximal promoter region was done, since the transcription rate of the class II gene is controlled by the well-conserved proximal promoter region. Six independent clones from PCR products of the HLA-DRA proximal promoter region and 16 clones from PCR products of the HLA-DRB proximal promoter region were isolated from the above cell lines and sequenced. Comparison of the nucleotide sequences of all 6 clones of DRA promoter showed that the sequences are extremely similar in both regulatory sequences and their intervening sequences. Sixteen clones of HLA-DRB promoter showed sequence variations such as substitution and insertion/deletion, and these 16 clones could be further grouped into 6 homologues with sequence homology. These data established that the melanoma cell lines studied here showed a differential susceptibility to IFN-γ on the modulation of HLA-DR molecules, that this modulation is transcriptionally regulated, and that the difference in promoter activity by sequence variation might contribute to such a differential transcriptional regulation at the promoter level.

Key Words: HLA-DR expression, IFN-γ, transcriptional regulation, malignant melanoma cell lines, proximal promoter region

INTRODUCTION

The class II major histocompatibility complex (MHC) encodes for cell surface glycoproteins that function as the fundamental element in the presentation of antigen to $CD4^+$ T lymphocytes.[1] The expression patterns of class II antigens are extremely diverse and tightly regulated. The class II molecules are expressed in a tissue-specific manner and the constitutive expression of class II molecules are mainly confined to cells of the immune system such as B lymphocytes, macrophages, dendritic cells, and some epithelial cells.[2] The expression of class II genes is also developmentally regulated and the appearance of these molecules can be found on precursors of various hemopoietic cell lineage and then be lost as cell differentiation proceeds.[3]

Many reports have indicated that transcriptional control is a major mode of regulation of class II genes. In most cases, it has been considered that the mRNA transcription rate of class II genes is responsible for the cell surface expression of class II molecules.[4-6]

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[2]Department of Microbiology, Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, Seoul, Korea
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Address reprint request to Dr. J.H. Park, Department of Microbiology, Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul 120-752, Korea. Tel: 82-2-361-5286, Fax: 82-2-392-7088, E-mail: jhpark5277@yumc.yonsei.ac.kr
[1]Current Address: Laboratory of Biochemical Immunogenetics, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY, 10021, USA

Several conserved *cis*-acting elements within the proximal promoter regions of class II genes, termed X, Y, and octamer regions, were also reported. These elements are critical for optimal class II gene expression in cell-specific constitutive expression and inducible expression of class II genes. Deletions or replacements of these elements reduce or completely abolish the promoter activity. Transcription of class II genes could also be controlled by several *trans*-acting nuclear proteins which interact with these conserved elements.[7]

Cytokines such as IFN-γ can increase the constitutive expression and also induce *de novo* class II expression in several class II negative cells.[8] Induced expression of HLA-DR molecules by IFN-γ, which is known to be the most potent inducer of HLA class II molecules, could be modulated transcriptionally either by *cis*-acting elements or by *trans*-acting elements.[9,10]

An unexpected but frequent expression of the HLA class II gene has been observed in neoplastic cells of various histologic origins.[11] In some HLA class II-negative melanomas, phenotypic modulation of HLA class II molecules can be achieved by stimulation with IFN-γ. Differential induction of the HLA class II gene by IFN-γ has been reported in melanoma cell lines, and this variable induction of these molecules by IFN-γ in tumor cells is transcriptionally regulated.[6,12,13]

Since these melanoma cell lines might represent valuable models to investigate transcriptional processes leading to class II gene expression, and in an attempt to evaluate the differential expression of HLA-DR, we have examined the expression of HLA-DR induced by IFN-γ in a panel of human melanoma cell lines, IGR3, Malme-3M, SK-Mel-24, and SK-Mel-28. We determined the surface expression and transcription rate of the HLA-DR gene, as well as analyzed the sequence of the HLA-DR proximal promoter region to establish a better understanding of the molecular mechanism of transcriptional regulation of HLA-DR expression.

MATERIALS AND METHODS

Maintenance of cell lines

Human malignant melanoma cell lines, Malme-3M (ATCC HTB64), SK-Mel-24 (ATCC HTB71), and SK-Mel-28 (ATCC HTB72) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and IGR3 was obtained from the Gustave-Roussy Cancer Institute (Villejuif, France).

IGR3, SK-Mel-24, and SK-Mel-28 cells were maintained in Eagle's minimum essential medium supplemented with 15 mM N-2-hydroxyethylpiperazine-N-ethane sulfonic acid (HEPES, Hazleton, Lenexa, KS, USA), 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal calf serum (Gibco BRL, Gaithersburg, MD, USA). Malme-3M cells were cultured in L-15 (Sigma Chemical Co., St. Louis, MO, USA) containing 15% fetal calf serum.

Monoclonal antibody

For the detection of surface expression of HLA-DR on melanoma cell lines, monoclonal antibody to HLA-DR, MAb B8.12.2 (mouse IgG2b kappa)[14] and FITC-conjugated goat anti-mouse antibody were obtained from Immunotech. S.A. (Marseilledex, France).

Flow cytometric analysis

Surface expression of the HLA-DR molecule was analyzed by indirect immunofluorescence and flow cytometry.[15] Cells were cultured for 4 hr, 24 hr, and 48 hr in the presence of recombinant human IFN-γ (Genzyme, Co., Cambridge, MA, USA) at a concentration of 200 U/ml. Cell suspension was washed with cold PBS three times, then incubated with the anti-HLA-DR monoclonal antibody for 30 min at 4°C. After washing with PBS, cells were incubated with FITC-conjugated goat anti-mouse antibody for another 30 min. After two washes, the cell pellet was resuspended in PBS. Flow cytometric analysis of HLA-DR molecules was carried out using a FACStar (Becton Dickinson, Mountainview, CA, USA).

Probes

cDNA probes for HLA-DRA[16] and -DRB gene[17] were obtained from Dr. SY Yang, Memorial Sloan-Kettering Cancer Center, NY, USA. Probes were radiolabeled with [α-^{32}P]-dCTP using a random primer labeling kit (Amersham Co., Arlington Heights, IL, USA) at a specific activity of $>10^8$ cpm/μg.

Northern blot hybridization

Total cellular RNA was extracted by guanidium isothiocyanate/cesium chloride method.[18] Twenty micrograms of total cellular RNA was electrophoresed through 1.2% agarose-formaldehyde denaturating gel and transferred to a nylon membrane (Schleicher & Schuell, Keene, NH, USA) using 20x SSC as de-

scribed.[19] Transferred RNA was fixed onto the membrane by UV cross-linking and prehybridized at 42°C for 4 hr using prehybridization buffer containing 5x SSC, 50% formamide, 5x Denhardt's solution and 100 μg/ml heat-denatured salmon sperm DNA. The blot was hybridized overnight at 42°C in hybridization buffer containing ^{32}P-labeled probe. Following two washes with 2x SSC, 0.5% SDS, once with 0.5x SSC, 0.5% SDS and then with 0.2x SSC, 0.5% SDS for 15 min each time at 65°C, the blot was dried and autoradiographed at −70°C for several days using Super HR-G film (Fuji Co., Tokyo, Japan).

PCR amplification and nucleotide sequencing analysis of PCR product

Genomic DNA from each cell line was extracted and the HLA-DR proximal promoter region was amplified by PCR. The *Hind* III tailed 5' and *Xba* I tailed 3' primers were used for amplificatinon of HLA-DRA and -DRB gene (Table 1). PCR was carried out in a 50 μl reaction volume containing 10 μl DNA, 50 mM KCl, 10 μM Tris-Cl, pH 8.3, 1.5 mM $MgCl_2$, 200 μM dNTP, 50 pmol primer set, and 1 U of *Taq* DNA polymerase (Perkin-Elmer Cetus Co., Emeryville, CA, USA). Amplification was accomplished with 25 cycles of denaturation at 94°C for 30 seconds, annealing of the primers at 60°C for 50 seconds, and DNA polymerization at 72°C for 30 seconds. The PCR products were analyzed by 2% agarose gel electrophoresis and photographed under UV transilluminator. The PCR product was purified, cut with *Hind* III and *Xba* I, and subcloned into pUC 18. Clones were sequenced using the dideoxy-chain-termination method.[20]

RESULTS

IFN-γ effects on the surface expression of HLA-DR

Four human melanoma cell lines, IGR3, Malme-3M, SK-Mel-24, and SK-Mel-28 were treated with 200 U/ml IFN-γ for 4, 24, or 48 hr and evaluated for the expression of HLA-DR on their surfaces by flow cytometric analysis. Fig. 1 shows the expression of HLA-DR molecules induced by IFN-γ in four melanoma cell lines. Before IFN-γ treatment, IGR3 and Malme-3M cells lacked expression of HLA-DR, while by contrast, a relatively high percentage (>50%) of SK-Mel-28 and almost (>90%) all of the SK-Mel-24 cells expressed HLA-DR (Fig. 2). The expression of HLA-DR molecules on Malme-3M and SK-Mel-28 cells was gradually induced after treatment with IFN-γ in both the expression rate and mean intensity by time-dependent manner. However, the expression rate and mean intensity of HLA-DR on SK-Mel-28 cells was higher than those of Malme-3M (Fig. 2 and 3). Regardless of IFN-γ treatment, all of the SK-Mel-24 cells and none of the IGR3 cell expressed HLA-DR (Fig. 1 and 2). These data show that melanoma cell lines used in our study are differentially modulated by IFN-γ in terms of the induced expression of HLA-DR molecules on their cell surfaces.

HLA-DR mRNA augmentation by IFN-γ

To test whether differential modulation by IFN-γ in melanoma cells as shown by FACS analysis resulted from differential control in the transcription rate, mRNA transcription of HLA-DRA and -DRB gene was analyzed by Northern blot hybridization. Cells from each cell line were cultured for 48 hr in medium with or without IFN-γ. There was no detectable expression of HLA-DR mRNA in the control sample of

Table 1. Primer Sequences for the HLA-DRA and -DRB Gene Proximal Promoter Region

Primer		Sequence (5'-3')	Expected size
HLA-DRA	Upstream	CCCAAGCTTGCCGTGATTGACTAACAG *Hind* III	271 (−236 ~ +35)
	Downstream	GCTCTAGAGCTCGGGAGTGAGGCAGA *Xba* I	
HLA-DRB	Upstream	CCCAAGCTTGCAACTGGTTCAAACCTT *Hind* III	248 (−222 ~ +26)
	Downstream	GCTCTAGAGCAAGTCTCACTCAGGGA *Xba* I	

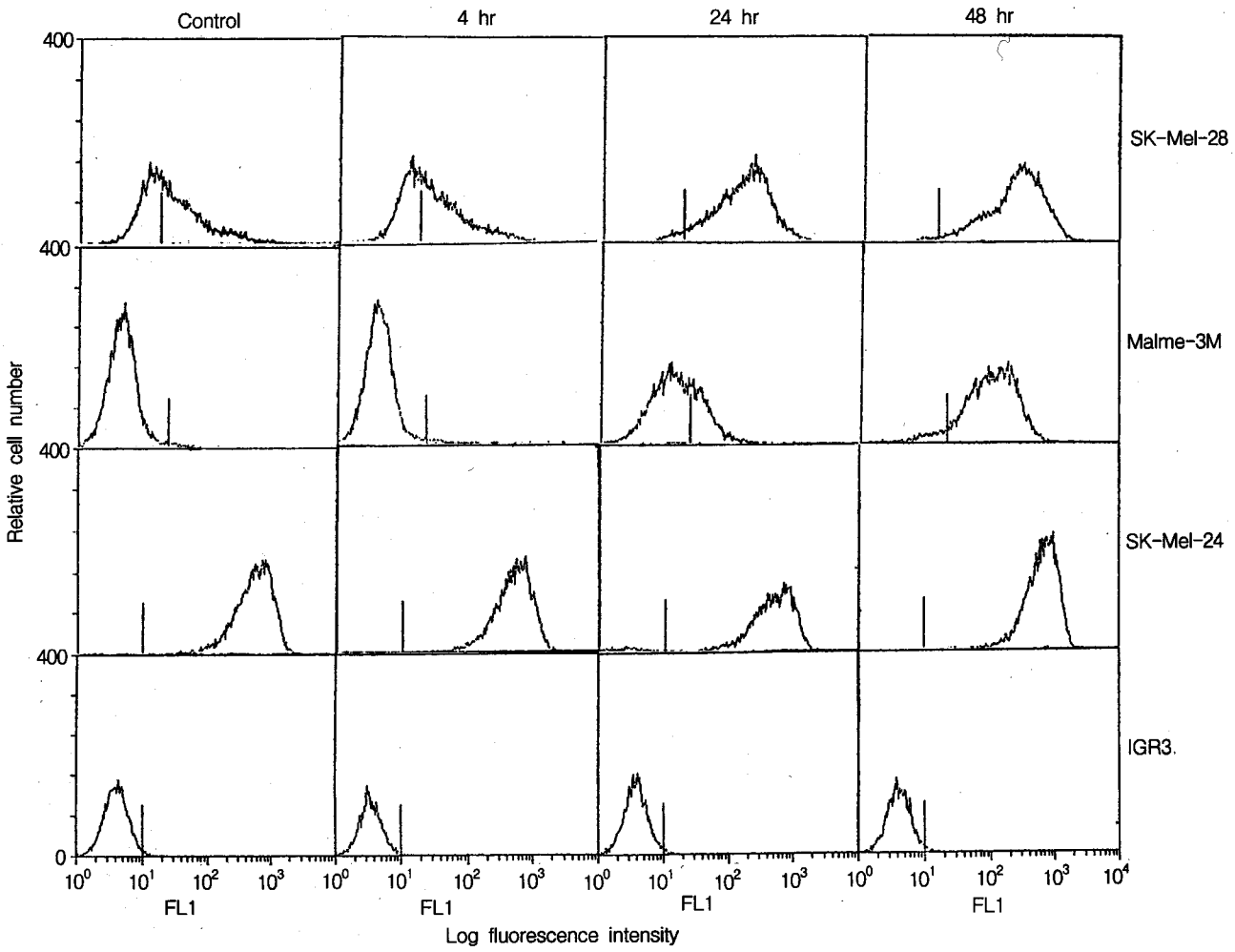

Fig. 1. FACS analysis of HLA-DR expression induced by IFN-γ in 4 different human malignant melanoma cell lines. Cells were treated with 200 U/ml IFN-γ for indicated time intervals or without (control) IFN-γ. Surface expression of the HLA-DR molecule on each cell line was analyzed by FACS histogram plotting fluorescence intensity vs cell number. First row, SK-Mel-28; 2nd row, Malme-3M; 3rd row, SK-Mel-24; 4th row, IGR3.

Malme-3M and SK-Mel-28. IFN-γ treatment induced HLA-DRA and -DRB mRNA in both cell lines, however SK-Mel-28 cells showed stronger band intensity than did the Malme-3M cells. Regardless of the IFN-γ treatment, SK-Mel-24 cells showed stronger band intensities in both HLA-DRA and -DRB mRNA than in any other cell lines. However, IGR3 expressed neither HLA-DRA nor -DRB mRNA (Fig. 4). These results suggest that the differential expression of HLA-DR molecules on the cell surface in these cell lines was transcriptionally controlled.

Sequencing analysis of HLA-DR promoter

Several conserved *cis*-acting elements within the proximal regions of class II genes termed X, Y, and octamer region were reported.[7] These elements are critical for the expression of optimal class II genes in cell-specific constitutive expression and inducible expression. To better understand the molecular mechanism of transcriptional regulation of HLA-DR molecules, it is important to evaluate as well as determine the sequence of the HLA-DR promoter. Therefore, the HLA-DRA and -DRB proximal promoter regions spanning −236 to +35 and −222 to +26 were amplified respectively by PCR and cloned into pUC 18, and sequencing analysis was done. All of the PCR products from each cell line showed the expected-size DNA, indicating that the differential transcription control in each cell line was not due to a defect in the proximal promoter region such as a large deletion of DNA (Fig. 5). To determine whether sequence variation of the *cis*-acting elements in the HLA-DR proximal promoter region might effect transcriptional regulation of the HLA-DR gene, sequencing analysis of PCR products of the HLA-DRA and -DRB prox-

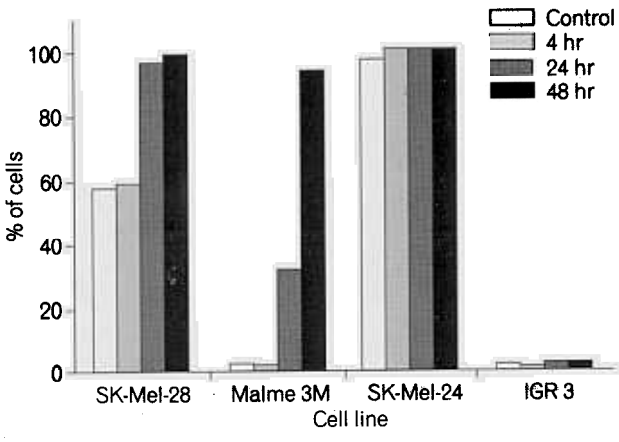

Fig. 2. Histogram shows the expression rate of HLA-DR in relation to the IFN-γ treated time intervals in each cell line.

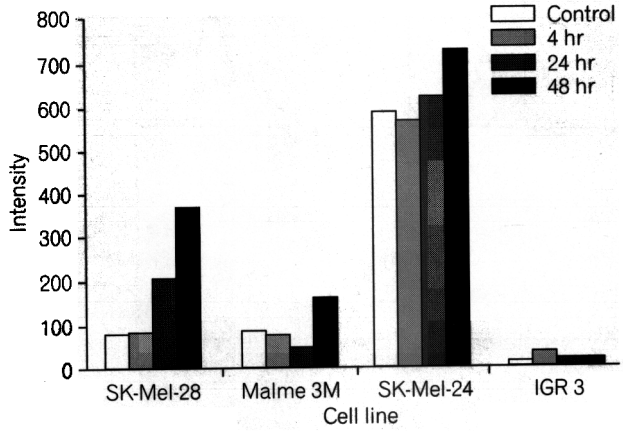

Fig. 3. Histogram shows mean fluorescence intensity of HLA-DR by the same experiment shown in Fig. 1. Intensity was plotted as mean channel number vs incubation time.

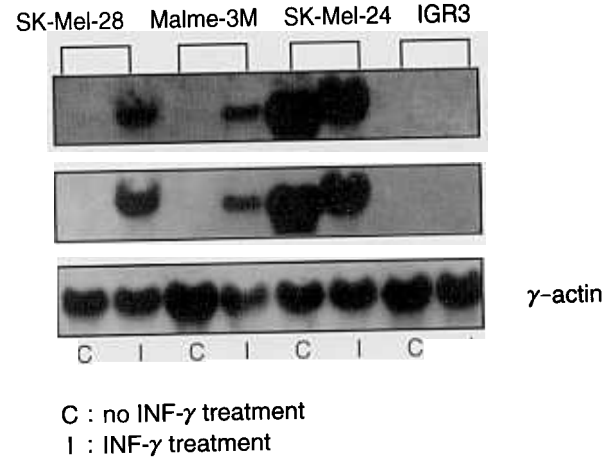

Fig. 4. Northern hybridization to examine HLA-DR mRNA transcripts before (C) and after (I) 48 hr treatment of IFN-γ in melanoma cells. Twenty μg of total cellular RNA was electrophoresed and transferred to a nylon membrane. Blot was hybridized with ^{32}P-labeled γ-actin probe and stripped, then hybridized with ^{32}P-labeled HLA-DRA and -DRB specific cDNA probe.

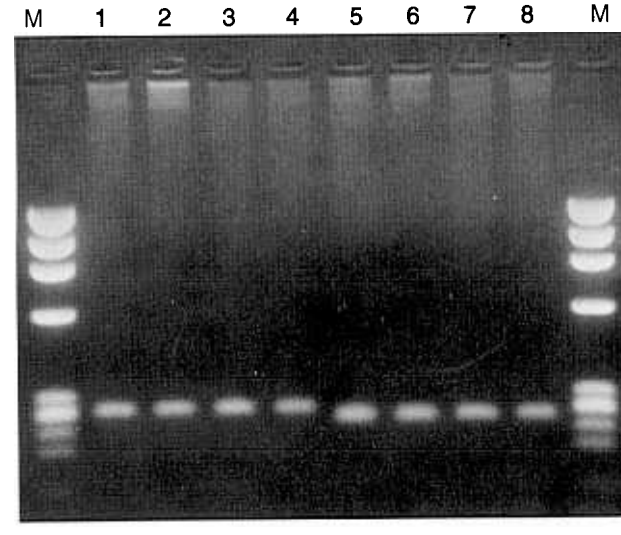

Fig. 5. Agarose gel electrophoresis of the PCR amplified HLA-DRA (lane 1-4) and -DRB (lane 5-8) proximal promoter region. M: DNA molecular weight marker, ϕx-174 RF DNA/Hae III. Lane 1-4 indicates the HLA-DRA proximal promoter region amplified with PCR of SK-Mel-28, Malme-3M, SK-Mel-24, and IGR3 respectively. Lane 5-8 indicates the HLA-DRB proximal promoter region amplified with PCR of SK-Mel-28, Malme-3M, SK-Mel-24, and IGR3 respectively.

imal promoter regions was done. At least 4 clones and 12 clones from each cell line respectively were isolated and sequenced for the analysis of the HLA-DRA and -DRB proximal promoter regions. After excluding clones showing identical sequences, 6 different clones and 16 different clones originating from the respective HLA-DRA and -DRB proximal promoter regions were finally collected and aligned (Fig. 6 and 7). Comparison of the nucleotide sequences of the HLA-DRA proximal promoter region of each cell line showed that the sequences were extremely similar in both regulatory sequences and their intervening sequences, except the only one bp variation in the X2 box in clone #1 originated from Malme-3M. The nucleotide sequence of the X2 box in clone #1 was TGCGCCA, hence those of the others were TGCGTCA. Other regulatory regions such as S, X1, and Y boxes and the octamer binding site were well conserved (Fig. 6). However, the nucleotide sequences of HLA-DRB proximal promoter region of each cell line were somewhat diverse, and only the S and X2 boxes were highly conserved (Fig. 7). These 16 clones could be further grouped into 6 clusters by sequence homology (Fig. 8). These 6 clusters showed sequence variation not only in the previously known *cis*-acting elements of the HLA-DRB proximal promoter region

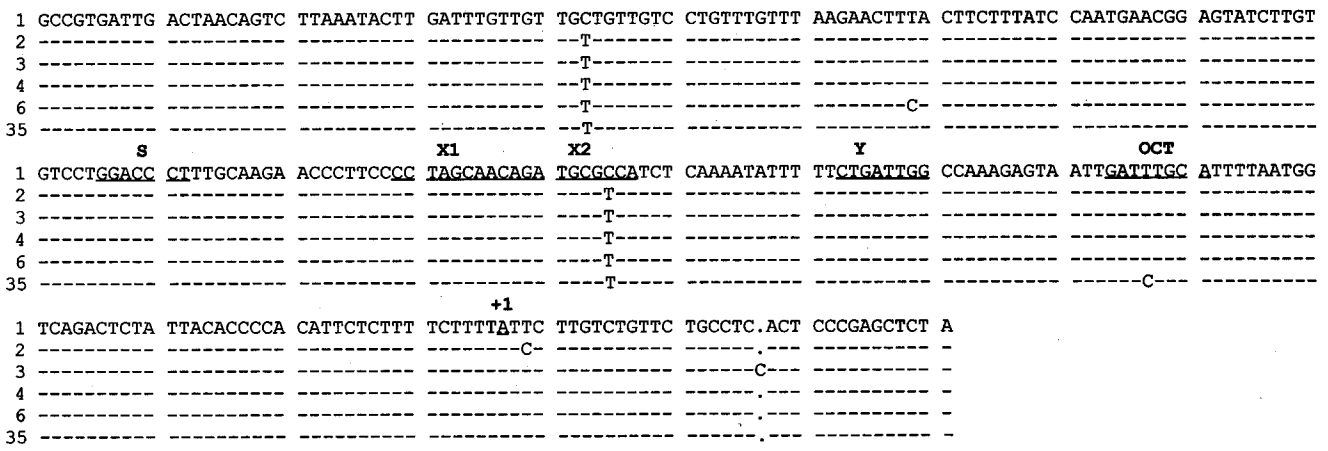

Fig. 6. Sequence analysis of HLA-DRA promoter of PCR product from 4 different human malignant melanoma cell lines. Malme-3M is represented by clone #1, 2, 3, and 4; SK-Mel-28: clone #4; SK-Mel-24: clone #4 and #6; IGR3: clone #4 and #35. Known cis-acting elements and their sequences are underlined. Dash (−) represents nucleotide identical to clone #1. Dot (.) indicates missing nucleotide. OCT, octamer binding site; +1, transcription initiation site.

such as X1 and Y boxes, but also in their intervening regions, suggesting that these sequence variations and their interaction with *trans*-acting factors might influence the differential transcription control in a constitutive state as well as in IFN-γ induction. The nucleotide sequences of the X1, X2, and Y boxes of cluster II, V, and VI were identical. This might indicate that these clusters are similar to each other. It seems likely that clusters III and IV are similar to each other, since the nucleotide sequences of these clusters are almost identical.

DISCUSSION

Class II genes of the major histocompatibility complex play a key role in regulating and restricting the immune response. They provide the molecular support for antigen presentation to T cells. Probably as a reflection of their regulatory role, class II gene products show a specific and limited cell-type distribution. The majority of mammalian cell types never display class II molecules, and expression is confined primarily to immunocompetent cells: B lymphocytes, macrophages, other antigen presenting cells (APC), and a few cells of diverse type in several tissues. A diverse collection of cells can also express class II genes under certain conditions. These are often specific epithelia-skin keratinocytes, intestinal vilar epithelium, thymic epithelium, vascular endothelium, kidney tubular cells, but they may also be non-epithelial cells such as astrocytes and melanocytes.[7] In most cases, these cells require IFN-γ to induce class II expression. However, several studies have shown their presence on cells with no immune function, including melanoma.[21,22] These expressions are increased by IFN-γ[6-13] Much evidence suggests that IFN-γ plays a role in the induction of HLA class II molecules on melanoma cells.[23-25] It has also been suggested that class II genes could be differentially regulated in some tumors such as melanomas, neuroblastomas and breast adenocarcinomas[6,12,13,26,27] This was based on the observation that there was an increase in steady-state mRNA and cell surface molecule expression of HLA class II genes following treatment with IFN-γ.

In this paper we have reported the effect of human recombinant IFN-γ on the expression of HLA-DR in human malignant melanoma cell lines. Four human malignant melanoma cell lines, IGR3, Malme-3M, SK-Mel-24, and SK-Mel-28, were used in this study. It seems likely that IFN-γ treatment did not affect HLA-DR expression in IGR3 cells. None of the IGR3 cells expressed HLA-DR in either the cell surface or mRNA transcript before or after treatment with IFN-γ. In contrast with IGR3, SK-Mel-24 cells fully expressed HLA-DR molecules before IFN-γ in terms of the constitutive level. In Malme-3M and SK-Mel-28 cells, IFN-γ induced a time-dependent increase in the level of expression of HLA-DR molecules in both the expression rate and mean intensity. However, SK-Mel-28 showed an increased expression of HLA-DR before and after treatment with IFN-γ than did Malme-3M. These differences in HLA-DR expression on the cell surface of melanoma cell lines coincided with HLA-DR mRNA levels in Northern hybridization. These suggested that the differential expression of HLA-DR molecules resulted from differ-

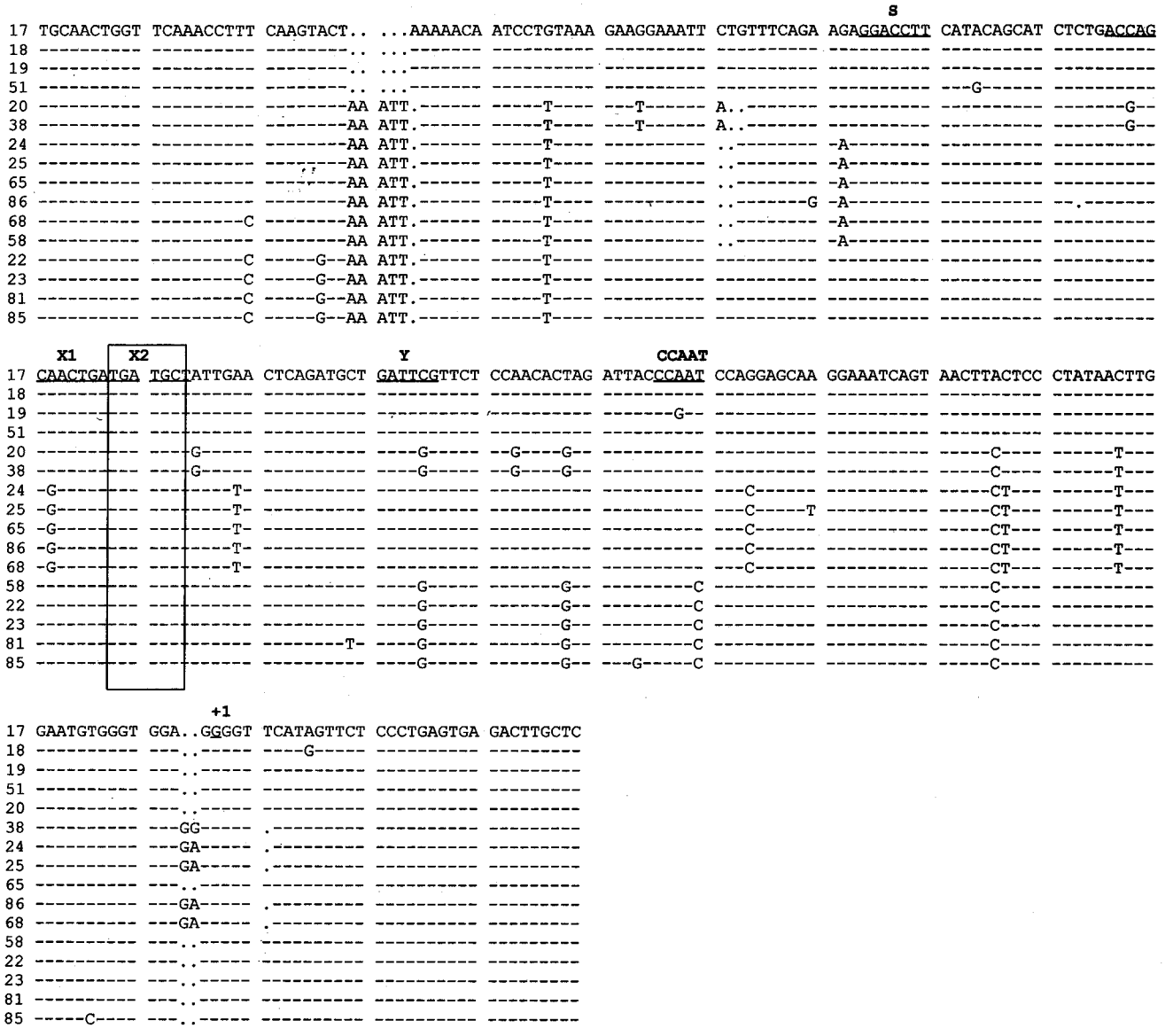

Fig. 7. Sequence analysis of HLA-DRB promoter of PCR product from 4 different human malignant melanoma cell lines. Malme-3M is represented by clone #17, 20, 22, and 38; SK-Mel-28: clone #24 and 25; SK-Mel-24: clone #18, 19, 20, 22, 23, 38, and 51; IGR3: clone #22, 24, 58, 68, 81, 85 and 86. Known cis-acting elements and their sequences are underlined. Dash (−) represents nucleotide identical to clone #17. Dot (.) indicates missing nucleotide. +1, transcription initiation site.

ential steady-state mRNA augmentation. Our results demonstrated that the melanoma cell lines studied showed a differential susceptibility to IFN-γ in HLA class II gene modulation, detected as cell surface expression and steady-state mRNA augmentation. As well, these events might be due to transcriptional regulation of the HLA-DR gene.

Regulated transcription depends on the interaction of diverse sequence-specific DNA binding proteins with various sequence motifs.[28] *In vitro* transcription and cell transfection studies allow analysis of DNA sequence elements responsible for the *cis*-control of class II gene expression. Strong promoter and enhancer activities have been demonstrated in the −180 to +1 regions. The promoter/enhancer activities exhibit several of the major features of class-II gene regulation, namely constitutive activity and inducibility by IFN-γ. They have been further characterized by 5' deletion analysis,[29] and by site-directed mutagenesis.[30] These analyses have shown the different class II proximal promoter regions to be of quite similar architecture. It was quite clear from the 5' deletion experiments that sequences 30 or 40 bp upstream of the X box play an important role in class II gene regulation in several cell types.[29] Mutations in the Z/W area (IRE) affect induction of transcrip-

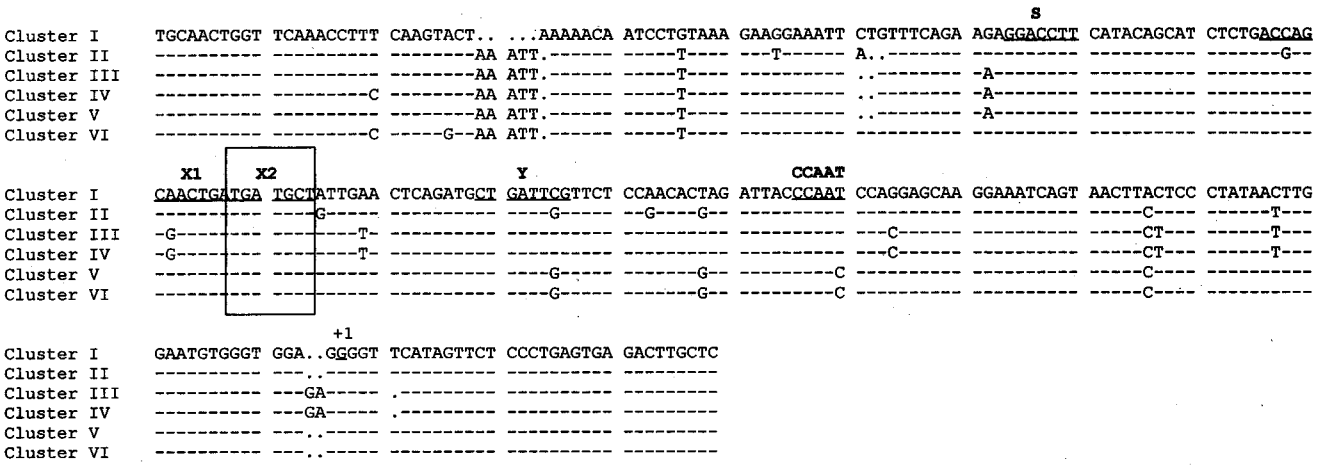

Fig. 8. *Sequence alignment of 6 HLA-DRB proximal promoter region homologues. Sixteen clones of Fig. 7 are clustered into 6 by their sequence homology. Cluster I: clone #17, 18, 19, and 51; Cluster II: clone # 20 and 38; Cluster III: clone #24, 25, 65, and 86; Cluster IV: clone # 68; Cluster V: clone #58; Cluster VI: clone # 22, 23, 81 and 85.*

tion by IFN-γ. The Z/W region actually contains several distinguishable sequence motifs, identified by sequence comparison and confirmed by functional analysis. The central role in this area of a sequence homology was originally pointed out by Servenius et al.[31] and referred to as the S box. The S motif lies 15−17 bp upstream from the X box. For DRA, mutations which affect the S box have strong "down" effects, almost as strong as X or Y box mutations.[32] The X-Y sequence resides in the −50 to −150 region of all MHC class II genes. The X and Y box motifs were the first control elements identified in MHC class II promoters and have been shown to be the focal point of class II gene regulation. The X and Y boxes are separated by a 19−20 bp stretch of conserved length but variable sequence. The X boxes from different genes show somewhat greater sequence diversity than do the Y boxes, often diverging from the consensus at three or four positions. The functional role of these motifs as enhancers does not seem to be IFN-γ inducible.[33] The activities of the X and Y boxes are clearly linked, as evidenced by the strong conservation of their relative distances. However, from transfection studies, it is not clear whether either motif can function in the absence of the other. Deletion or mutation of the Y box in the promoter renders it essentially inactive.[30] An octamer motif is present downstream from Y in the DRA promoter region. The octamer motif seems important but is apparently unique to DRA.[34,35]

The sequence information obtained in our study, together with the sequence of previously described studies permitted us to evaluate the transcriptional regulation of HLA-DR related to IFN-γ response. The nucleotide sequences of the HLA-DRA proximal promoter region of each cell line are quite similar to each other, however those of the HLA-DRB proximal promoter region of each cell line studied are somewhat polymorphic to each other even in the same cell line. Molecular cloning and sequence analyses of the HLA-DRB promoters of PCR products of 4 human malignant melanoma cell lines have revealed that the human genome contains 6 homologues of the DRB proximal promoter region. These 6 DRB homologues showed sequence variation such as substitution and insertion/deletion in known *cis*-acting elements and their intervening regions. It has been reported that mutation of the X1 box, or Y1 box alone,[32] or single base pair substitutions of both the X1 and X2 boxes[36] markedly reduced or altered HLA-DR expression in B cells. Our preliminary experiment revealed that clusters II, V, and VI, whose nucleotide sequences of the X1, X2, and Y boxes are identical, showed better factor-binding patterns than others (Lee et al. unpublished observation). This might suggest that these sequence variations and their interaction with *trans*-acting factors might influence the differential transcriptional regulation.

We demonstrated here the differential susceptibility of the HLA-DR gene to induction by IFN-γ and the sequences of the HLA-DR proximal promoter region. The sequence information, together with the surface expression and transcription rate of the HLA-DR gene, permitted us to evaluate the differential induction of HLA-DR molecules by IFN-γ in different melanoma cell lines. In conclusion, our study identified a distinct difference in the level of expression of HLA-DR in different melanoma cells. IFN-γ, the major regulator of class-II expression, increased the level of class-II-specific mRNA, suggesting that

the primary control of differential susceptibility of the HLA-DR gene induced by IFN-γ in melanoma cell lines lies at the level of transcription. The analysis of nucleotide substitutions and insertions/deletions described above did not provide clues for verifying the mechanism of the differential modulation by IFN-γ. Therefore, no obvious conclusion emerges from the comparison of the nucleotide sequences of the HLA-DR proximal promoter region. For further detailed analysis of the DRB promoter and the mechanism of differential modulation of the HLA-DR gene by IFN-γ, it would be necessary to perform the electrophoretic mobility shift assay using the diverse DRB proximal promoter region cloned in our study as probes and the nuclear extracts prepared from cell lines used in our study before and after treatment with IFN-γ.

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