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Patterns of constitutive and IFN-γ inducible expression of HLA class II molecules in human melanoma cell lines

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Abstract Major histocompatibility complex (MHC) class II proteins (HLA-DR, HLA-DP and HLA-DQ) play a fundamental role in the regulation of the immune response. The level of expression of human leukocyte antigen (HLA) class II antigens is regulated by interferon-γ (IFN-γ) and depends on the status of class II trans-activator protein (CIITA), a co-activator of the MHC class II gene promoter. In this study, we measured levels of constitutive and IFN-γ-induced expression of MHC class II molecules, analysed the expression of CIITA and investigated the association between MHC class II transactivator polymorphism and expression of different MHC class II molecules in a large panel of melanoma cell lines obtained from the European Searchable Tumour Cell Line Database. Many cell lines showed no constitutive expression of HLA-DP, HLA-DQ and HLA-DR and no IFN-γ-induced increase in HLA class II surface expression. However, in some cases, IFN-γ treatment led to enhanced surface expression of HLA-DP and HLA-DR. HLA-DQ was less frequently expressed under basal conditions and was less frequently induced by IFN-γ. In these melanoma cell lines, constitutive surface expression of HLA-DR and HLA-DP was higher than that of HLA-DQ. In addition, high constitutive level of cell surface expression of HLA-DR was correlated with lower inducibility of this expression by IFN-γ. Finally, substitution A→G in the 5′ flanking region of CIITA promoter type III was associated with higher expression of constitutive HLA-DR ($p<0.005$). This study yielded a panel of melanoma cell lines with different patterns of constitutive and IFN-γ-induced expression of HLA class II that can be used in future studies of the mechanisms of regulation of HLA class II expression.

Keywords HLA class II · Melanoma · CIITA · Polymorphism

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Introduction

Major histocompatibility complex (MHC) class I and II products play a central role in immune system function by restricting T cell recognition of foreign antigens (Ags) processed as small peptides. In many cases, the aim of cancer immunotherapy is to stimulate T cell responses against tumour-specific proteins. In the past, CD8+ cytolytic T lymphocytes (CTLs) were implicated as the main

effector cells in anti-tumour T cell responses. Numerous MHC class I-binding peptides derived from tumour-specific Ags have now been identified, and many have been used in clinical trials. Although CTLs can act alone in the effector phase of the immune response, it has become clear that priming of naive CD8+ T cells by antigen-presenting cells (APCs), including dendritic cells (DCs) (Bennett et al. 1998) and especially their ability to differentiate into long-lived memory cells, is dependent on "help" from CD4+ T cells. CD4+ T cells that recognize MHC class II-restricted epitopes encoded by cancer Ags, such as NY-ESO-1 (Jager et al. 2000; Zeng et al. 2001), MAGE (Tatsumi et al. 2002), TRP2 (Paschen et al. 2005) and CAMEL (Slager et al. 2003), have been detected in the peripheral blood of cancer patients, strongly suggesting that these tumour Ags are processed and presented in the MHC class II pathway in vivo. In cancer patients, however, tumour Ag-specific CD4+ T cells are frequently of the Th2 subtype, producing cytokines such as IL-4, IL-5 and IL-13, whereas IL-2- and interferon-γ (IFN-γ)-producing Th1 cells are believed to be required for proper activation of CD8+ effector T cells (Slager et al. 2003; Tatsumi et al. 2002). Therefore, induction of tumour-specific CD4+ Th1 cells should be also addressed in the development of immunotherapy for cancer. Mature DCs that express high levels of MHC and costimulatory molecules are required for the induction of Th1 immunity and potent CD8+ T cell-mediated anti-tumour responses.

MHC class II molecules are transmembrane glycoproteins found primarily at the surface of specialized cells of the immune system. They are heterodimeric molecules composed of α and β chains encoded by separate A and B genes. In humans, there are three types of MHC-II dimers called HLA-DR, HLA-DP and HLA-DQ. All MHC-II genes are clustered together in the class II region of the MHC locus on the short arm of chromosome 6 (Krawczyk and Reith 2006). Their expression is largely restricted to thymic epithelial cells (TECs) and to cells specialized in the capture and presentation of extracellular antigens (APCs) such as B cells, cells of monocyte–macrophage lineage and DCs (Reith et al. 2005). There are two general cell-type-specific modes of MHC-II expression: constitutive and inducible. Constitutive expression is mainly restricted to TECs and APCs. Other types of cells do not usually express MHC-II molecules, although they can often be induced to express MHC-II molecules after exposure to IFN-γ and other cytokines. Both constitutive and inducible MHC-II expression can be modulated in a cell-type-specific manner by various secondary stimuli.

The major regulator of MHC class II expression is class II trans-activator protein (CIITA). CIITA creates the scaffold required for a correct conformation of the MHC class II gene promoters and their transcriptional activation (Zhu et al. 2000) and activates both constitutive and inducible transcription of human leukocyte antigen (HLA) class II genes (Harton and Ting 2000; Steimle et al. 1993). CIITA is normally driven by four different cell-type-specific and inducible promoters: pI, pII, pIII and pIV (Muhlethaler-Mottet et al. 1997). pI is constitutively active in conventional DCs but can also be activated by IFN-γ in monocytes, macrophages and microglial cells. pIII is used preferentially by B cells, activated human T cells and plasmacytoid DCs. pIV is activated in TECs and responds to stimulation by IFN-γ in various cell types (Krawczyk and Reith 2006). In humans, a −168A-G polymorphism in the type III promoter of MHC class II transactivator (MHC2TA) has been associated with differential MHC class II expression (Swanberg et al. 2005).

There is considerable information on the distribution of MHC class I antigens in human tissues (Garrido et al. 1993, 1997; Garcia-Lora et al. 2001; Piskurich et al. 1999) but much less on the distribution of MHC class II antigens. The objectives of the present study were to investigate the patterns of constitutive and inducible expression of HLA class II and to explore the association of the transcription level of CIITA and polymorphism of CIITA promoter III with the expression of different MHC class II molecules in a panel of melanoma cell lines.

Materials and methods

Cell lines and cell culture

Cell lines were obtained from the European Searchable Tumour Cell Line Database (ESTDAB project, contract no. QLRI-CT-2001-01325, http://www.ebi.ac.uk/ipd/estdab; Pawelec and Marsh 2006). Samples were frozen in fetal calf serum (FCS) with dimethyl sulfoxide and stored in liquid nitrogen until used in experiments. Melanoma cell lines were grown in Roswell Park Memorial Institute (RPMI) medium (Biochrom KG, Berlin, Germany) supplemented with 10% FCS (Gibco BRL, Life Technologies, Karlsruhe, Germany), 2% glutamine (Biochrom KG) and 1% penicillin/streptomycin (Biochrom KG) at 37°C in a humidified atmosphere with 5% CO_2. To analyse IFN-γ-mediated inducibility of HLA class II expression in some experiments, cells were treated with IFN-γ (800 U/ml) for 48 h.

Cell surface expression of HLA-DR/DP/DQ was analysed on 42 cell lines. In addition, another group of 78 cell lines was analysed for CIITA-pIII polymorphism. Among them, 40 cell lines with positive constitutive HLA-DR expression were used to study an association of the level of HLA-DR expression and CIITA genotype. Out of the large panel of received melanoma cell lines, we selected an additional group of HLA-DR-positive and -negative mela-

noma cell lines (n=40) for analysis of mRNA expression levels of CIITA.

HLA II cell surface expression

Surface expression of HLA class II antigens was determined by indirect immunofluorescence using monoclonal antibodies GRB-1 (HLA-DR), B7-21 (HLA-DP) and SPVL3 (HLA-DQ; all from Immunotech, Beckman Coulter, Fullerton, CA, USA) on 42 melanoma cell lines. Cells (10^5) were washed three times in phosphate-buffered saline (PBS) then incubated with the monoclonal antibodies for 30 min at 4°C. Excess antibody was removed by washing cells with ice-cold PBS. Cells were stained with 1 μg of fluorescein-isothiocynate-labelled rabbit anti-mouse-($F(ab')_2$) immunoglobulins (Cappel, West Chester, PA, USA), washed again in PBS and analysed on a FACSort flow cytometer (Becton-Dickinson, San Jose, CA, USA). The results are expressed as mean fluorescence intensity (MFI).

Allelic discrimination PCR

DNA from the melanoma cell lines and 676 healthy controls were isolated using FlexiGene DNA Kit (Qiagen). Single nucleotide polymorphisms (SNPs) of MHC2TA/CIITA (A>G) (rs3087456) isoform III were typed. Genotyping of SNTPs was carried out using a Custom TaqMan SNP Genotyping Assays method (Applied Biosystems, Foster City, CA, USA). Minor groove-binding (MGB) probes were labelled with the fluorescent dyes VIC and FAM, respectively. Allelic discrimination using TaqMan® was performed using 10 ng of sample DNA in a 25-μl reaction containing 12.5 μl TaqMan® Universal PCR Mix (Applied Biosystems; PCR, polymerase chain reaction), 300 nM primers and 200 nM TaqMan® MGB probes (Applied Biosystems). Reaction conditions consisted of pre-incubation at 50°C for 2 min, 95°C for 10 min and then cycling for 40 cycles of 95°C for 15 s and 60°C for 1 min. Amplifications were performed in an ABI Prism 7750 machine (Applied Biosystems) for continuous fluorescence monitoring.

Statistical analysis

Data analysis was done using Statistical Package for the Social Sciences (SPSS) 12.0 software. Analysis of the correlation between the MFI of the constitutive expression of HLA-DR and of the transcription level of CIITA was performed using Student's t-test. For evaluation of the correlation between HLA-DR expression and CIITA genotype, we employed the analysis of variance and T_3 of Dunnett tests. Genotype frequencies were compared between melanoma cell lines and controls using the Yates corrected Pearson's χ^2 test for the 2×2 tables of the Fisher's exact test when appropriate. Odds ratios and corresponding 95% confidence intervals were calculated.

RT-PCR analysis

Total cellular RNA was isolated using a Qiagen RNeasy kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Total RNA (0.5 μg) was subjected to reverse transcription, and one tenth of the reaction mixture was used for PCR analysis. First-strand cDNA was synthesized with 2 μg of total RNA using Sensiscript reverse transcriptase (RT) kit (Qiagen) with 40 U/μl of RNasin (Promega, Madison, WI, USA) and random primers (Promega) in a final volume of 20 μl. The reaction was incubated at 42°C for 60 min and stopped at 95°C for 5 min. Transcriptional expression of CIITA was studied in a TaqMan® Gene Expression Assay using a CIITA probe (Hs001172106_m1; 5′-ATGCCAA TATCGCGGAACTGGACCA-3′) and beta glucuronidase (GUSB) endogenous controls (Applied Biosystems). Results were analysed in an ABI PRISM 7500 Real Time PCR System (Applied Biosystems) with a two-step PCR protocol (95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min) using 7500 Fast System Software 1.3.1. Expression levels of the CIITA gene were presented relative to GUSB levels.

Results

Constitutive expression of HLA class II in melanoma cell lines

Analysis of the distribution of different patterns of constitutive HLA class II expression (DR/DP/DQ isotypes) in 42 melanoma cell lines revealed that a large number of the cell lines (n=24) were negative for HLA-DP, HLA-DQ and HLA-DR (Table 1), whereas eight cell lines were positive for all three isotypes (Table 1). In addition, other eight cell lines were positive only for HLA-DR and HLA-DP but negative for HLA-DQ (Table 1). Finally, two cell lines were positive only for HLA-DP (ESTDAB-133 and ESTDAB-070; Table 1).

IFN-γ-induced expression of HLA class II in melanoma cell lines

Out of 24 melanoma cell lines with no constitutive expression of all of the three HLA class II isoforms, 9 cell lines showed induced expression of only DR and DP isoforms, whereas in 12 cell lines, IFN-γ treatment failed to induce any of class-II isoforms. In two melanoma cell lines, ESTDAB-140 and ESTDAB-073, only HLA-DR expression was increased in

Table 1 Patterns of constitutive and IFN-γ inducible expression in ESTDAB melanoma cell lines

Basal cell lines	HLA-DR	HLA-DP	HLA-DQ	IFN-γ treatment			
				HLA-DR	HLA-DP	HLA-DQ	
A (*n*=24)							
ESTDAB-004	−	−	−	−	−	−	
ESTDAB-159	−	−	−	−	−	−	
ESTDAB-038	−	−	−	−	−	−	
ESTDAB-109	−	−	−	−	−	−	
ESTDAB-049	−	−	−	−	−	−	
ESTDAB-081	−	−	−	−	−	−	*n*=12
ESTDAB-094	−	−	−	−	−	−	
ESTDAB-127	−	−	−	−	−	−	
ESTDAB-195	−	−	−	−	−	−	
ESTDAB-179	−	−	−	−	−	−	
ESTDAB-048	−	−	−	−	−	−	
ESTDAB-102	−	−	−	−	−	−	
ESTDAB-199	−	−	−	+	+	−	
ESTDAB-084	−	−	−	+	+	−	
ESTDAB-110	−	−	−	+	+	−	
ESTDAB-112	−	−	−	+	+	−	
ESTDAB-196	−	−	−	+	+	−	*n*=9
ESTDAB-200	−	−	−	+	+	−	
ESTDAB-146	−	−	−	+	+	−	
ESTDAB-020	−	−	−	+	+	−	
ESTDAB-069	−	−	−	+	+	−	
ESTDAB-162	−	−	−	+	+	+	*n*=1
ESTDAB-140	−	−	−	+	−	−	*n*=2
ESTDAB-073	−	−	−	+	−	−	
B (*n*=8)							
ESTDAB-152	+	+	+	++	++	++	*n*=8
ESTDAB-136	+	+	+	++	++	++	
ESTDAB-137	+	+	+	+	++	++	
ESTDAB-183	+	+	+	++	++	++	
ESTDAB-108	+	+	+	++	++	++	
ESTDAB-016	+	+	+	++	++	++	
ESTDAB-130	+	+	+	++	++	++	
ESTDAB-167	+	+	+	++	++	++	
C (*n*=8)							
ESTDAB-071	+	+	−	++	++	−	*n*=7
ESTDAB-206	+	+	−	++	++	−	
ESTDAB-058	+	+	−	++	++	−	
ESTDAB-153	+	+	−	++	++	−	
ESTDAB-184	+	+	−	++	++	−	
ESTDAB-041	+	+	−	++	++	−	
ESTDAB-074	+	+	−	++	++	−	
ESTDAB-129	+	+	−	++	++	++	*n*=1
D (*n*=2)							
ESTDAB-070	−	+	−	+	++	−	*n*=1
ESTDAB-133	−	+	−	+	++	+	*n*=1

response to IFN-γ. Finally, in just one melanoma cell line, ESTDAB-162, IFN-γ induced the expression of all three HLA class II isotypes (Table 1; Fig. 1).

All melanoma cell lines constitutively expressing HLA-DP, HLA-DQ and HLA-DR (*n*=8) were found to respond to IFN-γ treatment by increasing the level of expression of all three isoforms (Table 1).

Among cell lines with constitutive HLA-DP and HLA-DR expression and negative HLA-DQ expression (*n*=8), seven cell lines showed a further increase in HLA-DP and

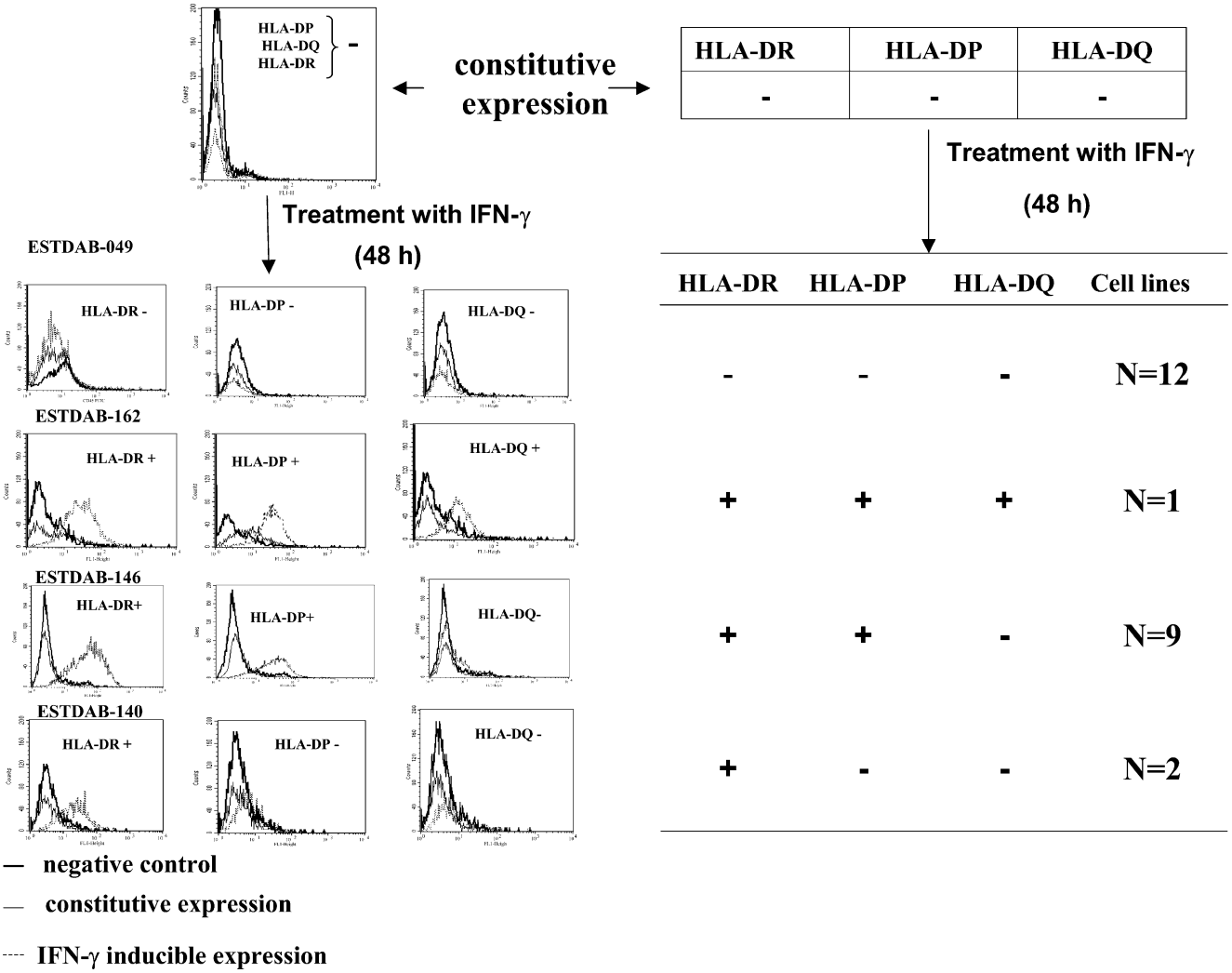

Fig. 1 Effect of IFN-γ (48-h treatment) on the HLA class II expression on cell surface of melanoma cells with negative constitutive expression of HLA-DR/DP/DQ. Half of these cell lines (12 out of 24) did not respond to IFN-γ treatment, nine cell lines showed IFN-γ-induced expression of HLA-DP and HLA-DR, two cell lines showed induction only of HLA-DR and only one cell line responded to IFN-γ treatment by increased expression of all three HLA class II isotypes

HLA-DR after IFN-γ treatment, although only one, ESTDAB-129, responded to IFN-γ with augmented expression of all of these isotypes (Table 1; Fig. 2).

Two cell lines, ESTDAB-133 and ESTDAB-070, which constitutively expressed only HLA-DP, showed different patterns of IFN-γ-induced MHC class II expression. Treatment of ESTDAB-133 with IFN-γ led to upregulation of HLA-DP, HLA-DR and HLA-DQ class-II subsets, whereas in ESTDAB-070, only HLA-DP and HLA-DR were upregulated by IFN-γ (Table 1; Fig. 3).

In summary, increased expression of different HLA class II isotypes in response to IFN-γ treatment showed the following hierarchy: HLA-DR > HLA-DP > HLA-DQ. The increased level of HLA class II molecules after IFN-γ treatment was lower in melanoma cell lines with a high level of HLA class II basal expression and higher in the melanoma cell lines with a low basal level of these molecules.

Correlation between HLA-DR surface expression and CIITA transcription level

We compared CIITA mRNA expression and HLA-DR cell surface expression in 40 melanoma cell lines. The MFI value was used to calculate the level of HLA-DR cell surface expression. A real-time quantitative RT-PCR was used to calculate CIITA expression. Melanoma cell lines were divided between those with low HLA-DR MFI (MFI<100, 28 cell lines) and those with high HLA-DR MFI (MFI>100, 12 cell lines). HLA-DR cell surface expression was found to be significantly

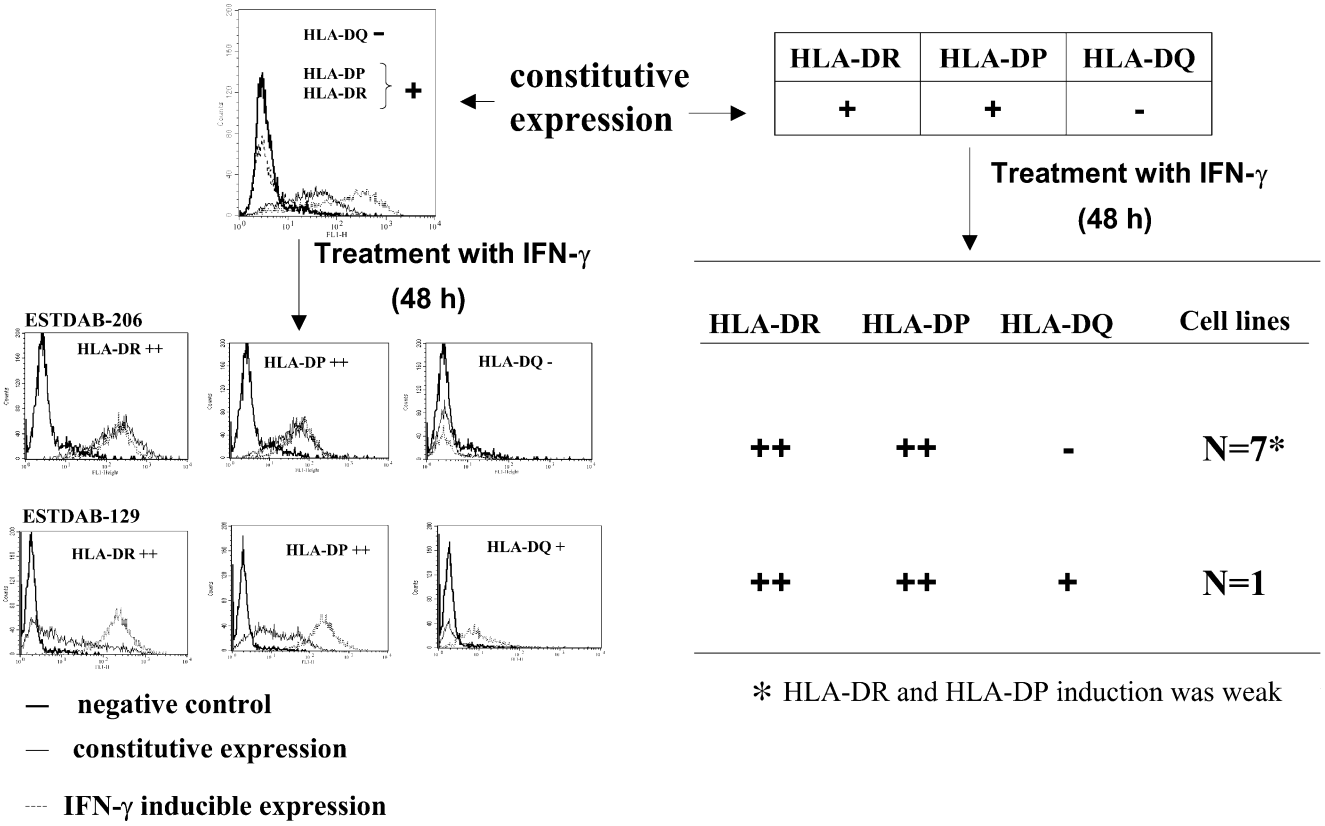

Fig. 2 Effect of IFN-γ (48-h treatment) on cell surface expression of HLA class II on melanoma cell lines with constitutive expression of HLA-DR and -DP (n=8). Seven cell lines responded to IFN-γ with an increase only in HLA-DR and HLA-DP expression, whereas one cell line showed an IFN-γ-induced increase in the expression of all three isotypes

correlated with CIITA transcription level (p=0.042; Fig. 4).

Association between HLA-DR expression and CIITA-pIII polymorphism

The polymorphism at position 5′ flanking region of the type III promoter of CIITA was studied in 40 melanoma cell lines and found to be associated with the level of HLA-DR surface expression in these cell lines. We found that 6 melanoma cell lines with the GG genotype have similar mean value of HLA-DR MFI to 17 melanoma cell lines with the AG genotype, and it is significantly higher than the values found in the remaining 17 cell lines with AA genotype (Fig. 5). A significant difference (p=0.020) was found between the HLA-DR MFI of AA genotype (lowest HLA-DR MFI) and AG genotype. However, there was no significant difference (p=0.186) between HLA-DR MFI of GG and AA CIITA genotypes (possibly because there were only six melanoma cell lines with GG CIITA genotype). When cases with AG and GG CIITA genotypes (the two genotypes with highest HLA-DR MFI) were considered as one group, the HLA-DR MFI was significantly different from that of the AA CIITA genotype (p=0.005). These data suggest that the presence of G at position −168 of the 5′ flanking region of type III CIITA promoter is associated with higher surface-constitutive expression of HLA-DR.

Allele frequencies for SNPs of the type III CIITA promoter were compared between melanoma cell lines and controls (n=676), and no significant differences were found (Table 2). Therefore, this polymorphism does not play an important role in melanoma.

Discussion

Expression of MHC class II was first reported on human melanoma cell lines by Wilson et al. (1979). Normal melanocytes, unlike Langerghans cells, did not express HLA class II antigens when studied by immunohistochemistry with either light or electron microscopy (van Duinen et al. 1984). Dysplastic nevi may show local expression of HLA class II antigens but only in about 20% of lesions (Bergman et al. 1988; Elder et al. 1989; Holzmann et al. 1987; Ruiter et al. 1982, 1984). HLA class II expression has been reported in 40–70% of primary cutaneous melanomas (Brocker et al. 1985; D'Alessandro et al. 1987; Elder et al. 1989; Holzmann et al. 1987; Ruiter et al. 1984; Thompson et al. 1982;

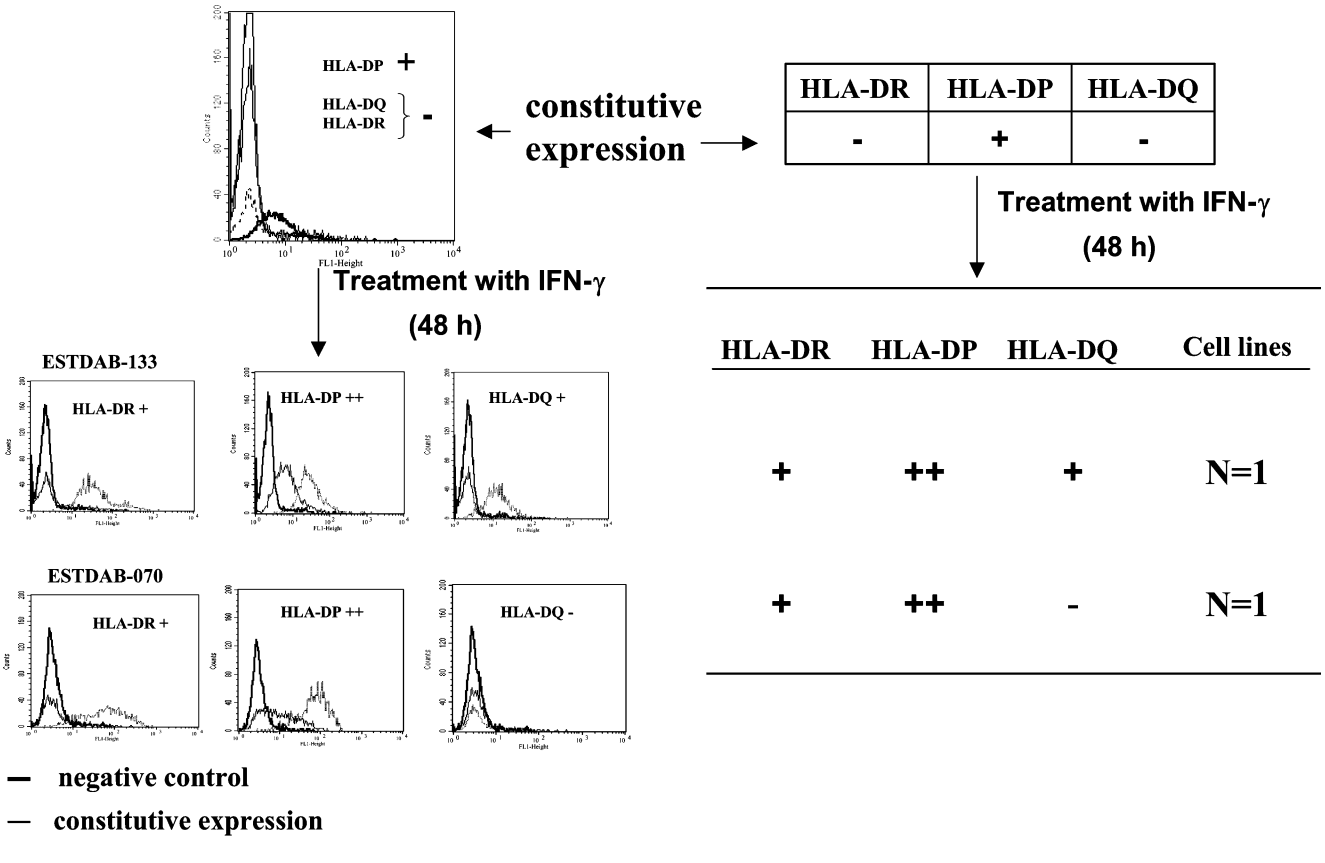

Fig. 3 Effect of IFN-γ (48-h treatment) on cell surface expression of HLA class II on melanoma cell lines with constitutive expression of HLA-DP (n=2). One cell line responded to IFN-γ with an increase only in HLA-DR and HLA-DP expression, whereas the remaining one showed IFN-γ-induced increase in the expression of all three isotypes

Zaloudik et al. 1988). The frequency of HLA class II expression in melanoma metastases depends on the stage of the lesion, being observed in around 80% of loco-regional metastases but only 10% of distant cutaneous metastases. In the present study, the type of lesions from which the melanoma cell lines had been established was not known. It was observed that constitutive expression of HLA-DR prevails over that of HLA-DP and HLA-DQ, as previously

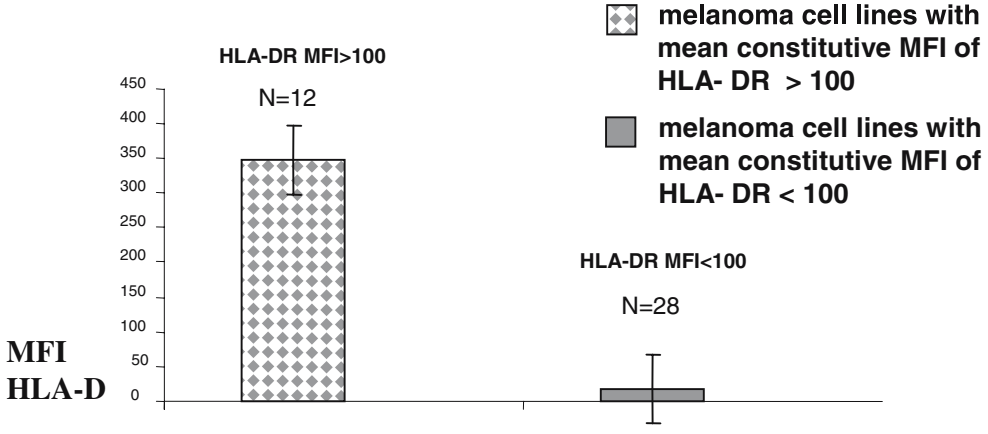

Fig. 4 High expression of HLA-DR (MFI>100) correlates with high levels of CIITA transcription (0.053) and vice versa. Total mRNA was isolated from 40 melanoma cell lines to study CIITA transcription level by RT-PCR analysis. In the same melanoma cell lines, HLA-DR constitutive expression was determined by flow cytometry (MFI). *MFI* Mean fluorescence intensity, *CIITA/GUSB* mean value of CIITA transcript copy numbers normalized against that of GUSB

MFI HLA-DR	Mean value of CIITA/GUS
>100	0.053
<100	0.012

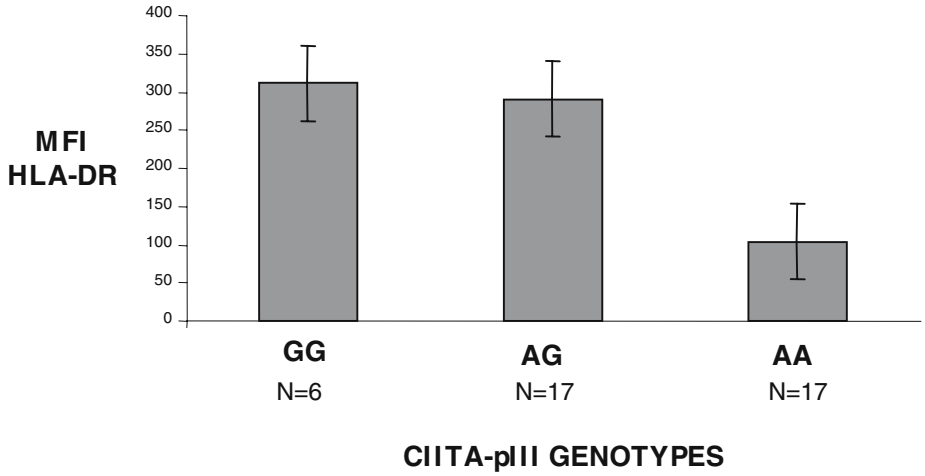

Fig. 5 Association between constitutive HLA-DR expression and CIITA-pIII polymorphism. **a** The mean fluorescence intensity (*MFI*) for the different CIITA-pIII genotypes. **b** Comparison of HLA-DR MFI between cell lines with CIITA-AA genotype and those with CIITA GG or GA genotypes considered together

CIITA-pIII Genotypes	MFI (±SD) of DR (GG and AG) / DR(AA)	p-value
GG/AG versus AA	296(±48) / 104 (±30)	0.005**

MFI: Mean fluorescence intensity.
* * Significant difference ($p<0.05$)
N - number of cell lines.

reported (D'Alessandro et al. 1987; van Vreeswijk et al. 1988). An association between HLA-DR expression and favourable prognosis has been observed in breast (Brunner et al. 1991), gastric (Hilton and West 1990) and colorectal carcinomas (Andersen et al. 1993) and squamous cell carcinoma of the larynx (Esteban et al. 1990). In melanoma, however, HLA-DR expression has been correlated with a more aggressive phenotype and high risk of metastasis (Brocker et al. 1985; Concha et al. 1991; Cabrera et al. 1995; Lopez-Nevot et al. 1988).

In our study, all melanoma cell lines that were positive for constitutive expression of HLA-DR were also positive for HLA-DP but not for HLA-DQ. Only two melanoma cell lines with constitutive expression of HLA-DP did not have constitutive expression of HLA-DR. The low frequency of HLA-DQ expression and low frequency of its induction by IFN-γ may be explained by a CIITA-independent pathway of expression of this isoform (Douhan et al. 1997; Ono et al. 1991).

IFN-γ induction of the CIITA gene is mediated by phosphorylation of signal transducer and activator of transcription 1 (STAT1), which promotes transcription of IFN regulatory factor-1 (IRF-1) and binding of both IRF-1 and STAT1 in association with upstream transcription factor 1 on proximal promoter IV (Muhlethaler-Mottet et al. 1998; Piskurich et al. 1999). We observed that 50% of melanoma cell lines (12 out of 24 cell lines) without constitutive HLA class II expression did not increase class II surface expression after IFN-γ treatment. This finding may be explained by a loss of CIITA expression resulting from DNA methylation (Holling et al. 2004; Morimoto et al. 2004; Nie et al. 2001; Satoh et al. 2004; van den Elsen et al. 2000; van der Stoep et al. 2002). CIITA is directly controlled by an IFN-γ-mediated signalling pathway involving JAK/STAT proteins. Therefore, defects in STAT1 activation might affect CIITA expression. Loss of STAT1 may prevent IFN-γ-induced HLA class II upregulation in these melanoma cell lines, similar to previous findings from our laboratory in a gastric adenocarcinoma cell line (Abril et al. 1998).

CIITA was originally identified by cloning a gene with a null mutation in hereditary MHC class II deficient (bare lymphocyte) syndrome (Steimle et al. 1993). It is a global regulator of the expression of proteins involved in antigen presentation and processing including MHC class II, CD74, HLA-DM and MHC class I molecules (Chang and Flavell

Table 2 Allele frequencies for SNPs of the type III CIITA promoter between melanoma cell lines and controls

MHC2TA	Patients, *n*=78 (%)	Healthy controls, *n*=676 (%)	*P*	OR
AA	35 (44.9)	356 (52.7)	0.19	0.73
AG	35 (44.9)	274 (40.5)	0.46	1.19
GG	8 (10.2)	46 (6.8)	0.26	1.57
A	105 (67)	986 (72.9)	0.13	0.76
G	51 (33)	366 (27.1)	0.13	1.13

1995; Chang et al. 1996; Kern et al. 1995; Nagarajan et al. 2002). We found a significant relationship between CIITA transcription level and HLA-DR constitutive surface expression on melanoma cell lines. Thus, melanoma cell lines with higher CIITA transcription had higher HLA-DR constitutive surface expression and vice versa. Earlier studies had reported that MHC class II expression in melanoma cells can be related to constitutive expression of CIITA, which mediates both constitutive and IFN-γ-inducible MHC class II expression in all other cell types (Goodwin et al. 2001). Furthermore, transgenic CIITA-deficient mice are totally devoid of cells expressing MHC class II molecules except for a subset of TECs (Chang et al. 1996).

The presence of distinct CIITA haplotypes across inbred strains of mice that differ in degree of MHC class II expression is consistent with the hypothesis that these differences in expression are produced by valuable functional polymorphisms in the 5′ flanking region of CIITA (Swanberg et al. 2005). In the present study, CIITA promoter-III polymorphism was related to differential expression of HLA-DR. Thus, the presence of G (GG/AG genotypes) at position −168 of 5′ flanking region of type III of CIITA promoter was significantly associated with a high level of HLA-DR constitutive expression. In addition, we observed that melanoma cell lines with the highest expression of HLA-DR demonstrate the lowest response after IFN-γ treatment. All this taken together suggests that, possibly, a substitution of −168A to G in the studied CIITA promoter SNP could be associated with low inducibility of HLA II to IFN-γ. These results are in agreement with the observations of other groups done in peripheral blood cells, where A→G substitution in 5′ flanking region of type III promoter of CIITA was associated with a lower induction of class II genes (Swanberg et al. 2005).

Finally, this study yielded a panel of melanoma cell lines with different patterns of constitutive and IFN-γ-induced expression of HLA class II that can be used in future investigations of the mechanisms of regulation of HLA class II expression.

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