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Distinct *MHC* Gene Expression Patterns During Progression of Melanoma

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Abstract

Abnormal expression of major histocompatibility complex (*MHC*) molecules in melanoma has been reported previously. However, the *MHC* molecule expression patterns in different growth phases of melanoma and the underlying mechanisms are not well understood. Here we demonstrate that in vertical growth phase (VGP) melanomas *MHC* genes are subject to increased rates of DNA copy number gains, accompanied by increased expression, in comparison to normal melanocytes. In contrast, *MHC* expression in metastatic melanomas drastically decreased compared to VGP melanomas, despite still prevalent DNA copy number gains. Subsequent investigations found that the master transactivator of *MHC* genes, *CIITA*, was also significantly down regulated in metastatic melanomas when compared to VGP melanomas. This could be one of the mechanisms accounting for the discrepancy between DNA copy number and expression level in metastatic melanomas, a potentially separate mechanism of gene regulation. These results infer a dynamic role of *MHC* function in melanoma progression. We propose potential mechanisms for the overexpression of *MHC* molecules in earlier stages of melanoma as well as for its down-regulation in metastatic melanomas.

INTRODUCTION

Melanoma, a malignant tumor that originates in melanocytes, is found predominantly in the skin. The stages of melanocytic progression are well documented. Dysplastic nevi first transform into melanoma in situ. Subsequently melanoma cells progress from the radial growth phase (RGP) to the vertical growth phase (VGP), and finally to the metastatic phase. Early detection is key in melanoma treatment. In the RGP phase, melanoma can be cured by surgical excision. However, if the progression of melanoma enters the VGP phase, it negatively impacts patient prognosis (Byers and Bhawan, 1998).

The genes in major histocompatibility complex (*MHC*), also called human leukocyte antigen (HLA), are clustered in a large genomic region on chromosome 6. This group of genes encodes heterodimeric antigen-presenting proteins, crucial for the proper function of the

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immune system. There are 3 major *MHC* Class I genes: HLA-A, HLA-B and HLA-C, and three major *MHC* Class II genes: HLA-DR, HLA-DQ and HLA-DP. *MHC* Class I molecules present peptides derived from cytosolic proteins and interact exclusively with CD8⁺ T cells, whereas Class II molecules present peptides derived from extracellular proteins and interacts exclusively with CD4⁺ T cells. *MHC* class I molecules are displayed at the surface of most cells. *MHC* class II molecules are constitutively expressed only in antigen-presenting cells, but can be induced to be expressed in other cell types in response to various stimuli of which IFN- γ is the most potent and well known. Expression of *MHC* genes is mediated by multiple transcription regulators, for example the class II transactivator (CIITA) is an essential regulator for the expression of Class II genes (Drozina et al., 2005).

Abnormalities in *MHC* molecules have been studied extensively in cancer. The fact that down-regulation of the *MHC* Class I molecules is frequently found in human cancer, including melanoma, and is often correlated with poor prognosis (Ogino et al., 2003, 2006; Meissner et al., 2005), suggests that it may contribute to escape of tumor cells from T-cell surveillance (Garrido et al., 1997; Koopman et al., 2000; Marincola et al., 2000; Ogino et al., 2003, 2006; Meissner et al., 2005; Norell et al., 2006; Chang and Ferrone, 2007). In contrast, solid tumors of different histotypes were reported to express Class II molecules (reviewed in Altomonte et al. (2003)) and selected tumor-associated antigens (TAA) were effectively presented by *MHC* Class II molecules to CD4⁺ T cells (Renkvist et al., 2001; Tatsumi et al., 2003). However, the functions of the *MHC* class II expression, as well as their clinical implications, are often controversial. For melanoma, earlier studies suggested that high expression of class II antigens were associated with shorter survival (van Duinen et al., 1988). In later studies, significant association of HLA class II antigen expression with longer survival was reported in melanoma patients (Anichini et al., 2006). Similar associations were also reported in large B-cell lymphoma and colorectal cancers (Rimsza et al., 2004; Matsushita et al., 2006).

HLA class I and class II protein expression was studied in the human melanoma cell line collection from the European Searchable Tumor Cell Line Database (ESTDAB) (Rodriguez et al., 2007; Mendez et al., 2008, 2009). In 67% of the cell lines, HLA class I expression was down-regulated via various mechanisms. HLA class II expression was found in a subset of the 42 cell lines studied, and there seemed to be positive correlation between class I and class II expression. Since immunologic events seem to be intimately involved in the clinical course of this disease (Mortarini et al., 2003), and no systematic study has been done to compare *MHC* class molecule expression in different stages of melanoma development, we used DNA and expression microarray data from an extended collection of melanoma cell lines established from patients to identify distinct patterns of expression of *MHC* and *MHC*-related genes in VGP and metastatic melanoma cells. Based on these results we propose potential mechanisms for the overexpression of *MHC* molecules in VGP and down-regulation of these molecules in metastatic melanoma relative to VGP.

MATERIALS AND METHODS

Cell Lines and Cell Culture

Normal melanocytes and primary melanoma cell lines were obtained from M. Herlyn (Wistar Institute, Philadelphia, PA). Human melanocytes and melanoma cells were maintained in media as described previously (Satyamoorthy et al., 2003) and were grown at 37°C and 10% CO₂.

Microarray Analysis for Gene Expression and Copy Number Changes

Microarray-based gene expression analysis was done using the Affymetrix HG-U133A chips (Affymetrix Inc., Sunnyvale, CA). Labeling and hybridization steps were carried out as described previously (Tsavachidou et al., 2004). All gene expression analyses were done using the R statistical programming language and several accompanying Bioconductor packages (Gentleman et al., 2004). First, raw cell files were subject to quantile normalization. Probes not expressed to a mas5 normalized intensity value of at least 100 in more than 25% of all samples were removed from the data set. To determine which genes appear differentially expressed between VGP and metastatic groups, every probe set was subject to a group-wise comparison with a t-test where the significance level was adjusted for multiple comparisons. All genes with a P -value < 0.05 were considered to be differentially expressed between groups. Microarray DNA copy number profiling was done for 52 melanoma cell lines. All cell lines were assayed on a microarray composed of 4,134 bacterial artificial chromosomes (BACs) and hybridization protocols described in detail by Naylor et al. (2005). Sample-wise data normalization was done under the assumption that the mean copy number of each melanoma was diploid. Subsequent data visualization and analysis was performed using the CGHAnalyzer software suite (Margolin et al., 2005). All primary and processed profiling data were deposited in NCBI's Gene Expression Omnibus repository for public access (Tsavachidou et al., 2004; Hoek et al., 2006; Greshock et al., 2009).

Ingenuity Analysis

The Ingenuity Pathway Analysis software (IPA, Ingenuity Systems, Mountain View, CA) was utilized to map genes into different pathways and for visualization. For copy number studies, gene amplification and deletion frequencies were input into Ingenuity. Significant 'nodes' were noted where alteration frequencies exceeded 20% (colored red and green in the case of DNA gains and losses respectively). For expression studies, Ingenuity input was limited to differentially expressed genes having fold changes of >1.5 between tumor stage groups. The significantly overexpressed genes are colored green and the significantly underexpressed genes are colored red.

Transcript Validation by Taqman Gene Expression Assays

HLA-DRA, CD74 and CIITA transcript levels relative to β -actin were measured in each melanoma cell line by one step real-time qPCR analysis using Applied Biosystems Prism (ABI) 7900HT Sequence Detection System as described previously (Mu et al., 2003). Taqman primers and probes for β -actin and HLA-DRA were designed using Primer Express software (Applied Biosystems, Foster City, CA). The sequences for each gene are: HLA-DRA-QF: GGGTCTGGTGGGCATCATTA, HLA-DRA-QR: TTCTGCTGCATTGCTTTTGC, HLA-DRA-QP: CAATCCCTTGATGATGAAGATGGTCCCA, H-ACTIN-QF: GCAAAGACCTGTACGCCAACA, H-ACTIN-QR: TGCATCCTGTCTGGCAATG, and H-ACTIN-QP: TGGCGGCACCACCATGTACCC. The CD74 and CIITA primers and probes were purchased from ABI gene expression assays collection. The assay ID for CD74 is Hs00269961_ml, and the assay ID for *MHC2TA* is Hs00172094_ml.

Western Blot Analysis

Protein levels were measured in all cell lines for CD74 and HLA by Western blot analysis. First, cells were lysed in M-PER Mammalian Protein Extraction Reagent (PIERCE, Rockford, IL) following manufacturer's protocol. Following SDS-PAGE and transfer onto a PVDF membrane (Millipore, Billerica, MA), the membrane was blocked in 5% milk in Tris-buffered saline containing 0.1% (v/v) Tween 20 (TBCT), and subsequently probed with

anti- CD74 (GeneTex Inc. San Antonio, TX) at 1:50 dilution, or anti-HLA (Abcam, Cambridge, MA) at 1:300 dilution. The catalog number for the Abcam antibody is ab8085, and recognizes HLA DR + HLA DP. GAPDH control antibody was purchased from Abcam and was used at 1:1000 dilution.

RESULTS

Frequent Copy Number Gains at Chromosome Arm 6p in Melanoma Cell Lines

The copy number status of all *MHC* loci was derived by CGH assay using the clone directly covering the respective region, or by identifying the nearest mapping clone. This was done using the ‘flanking regions’ function implemented by the CGHAnalyzer software. Copy number gains were defined where clone’s $\log_2$ copy number exceeded 0.25, while losses were defined when the clone’s $\log_2$ copy number was less than -0.25. A total of 95 melanomas (52 cell lines (Fig. 1A and Supplemental Table S1) and 43 tumor tissues (Fig. 1B and Supplemental Table S2)) were analyzed for DNA copy number changes (Greshock et al., 2009). Copy number gains of *MHC*-I and *MHC*-II (6p21.32) are common in melanomas, where 32.7% (17/52) of cancer cell lines and 25.6% (11/43) of tumor tissues harbor a copy number gain. In all but 3 cases (1 cell line, 2 tumor tissues), the copy number gains were $< \sim 5$ copies ($\log_2$ ratio 0.25-0.65). There were no significant differences in copy number gain frequency when stratifying by the presence of *BRAF* or *NRAS* mutations (Fig. 1).

Correlation of *MHC* Gene Amplification and Overexpression in VGP Melanoma Cell Lines

To assess concurrent aberrations at both DNA and RNA level for different canonical pathways in melanomas, with the specific emphasis on the antigen-presenting pathway, we analyzed genome-wide microarray based gene expression profiling data for a subset of cell lines described above, including 5 melanocytes, 13 VGP cell lines and 18 metastatic cell lines (Supplemental Table S3) (Tsavachidou et al., 2004; Hoek et al., 2006). Significantly differentially expressed genes were cross-tabulated for each pair of diagnosis groups. This includes comparisons of VGP melanoma lines and melanocytes, metastatic melanoma and melanocytes, and metastatic and VGP. The deletion and amplification frequencies calculated by aCGH data were aligned with differentially expressed gene lists from expression microarray data and imported into the Ingenuity pathways analysis software. For VGPs, *MHC* genes located on 6p21-22 showed frequent copy number gains. In addition to *MHC*-II α , *MHC* II- β , and *MHC*-I α genes, these also include *LMP2*, *LMP7*, *TAP1*, *TAP2*, and *TPN* (Supplemental Fig. 1). In contrast, *MHC*-I β molecules like B2M, which maps to 15q21, were not gained (and even deletion was observed in some cell lines). Also *CLIP* (CD74 antigen, *MHC* II- γ chain), which is located on 5q32, did not show frequent copy number gains. The same genes that showed copy number gains on chromosome 6, including *MHC*-II α genes, *MHC*-II β genes, *MHC*-I α genes and *LMP2*, *LMP7*, *TAP2*, were all overexpressed in VGP cell lines when compared to normal melanocytes as well (Fig. 2A and Table 1). In contrast, *MHC*-I β genes, which are not located on chromosome 6, did not show copy number gains and were also not up-regulated in VGP. The exceptions are *TAP1*, which was not overexpressed despite frequent copy number gains in VGP, and *CLIP*, which is on 5q32 and did not show DNA copy number alterations but was significantly up-regulated in VGP. Overall, however, there is a good correlation between the copy number gains on chromosome 6 and the enhanced expression of the *MHC* gene cluster for VGP melanoma cell lines.

Expression of *MHC* Genes is Down-Regulated in Metastatic Melanoma Cell Lines Compared to VGP Lines

In contrast to VGP cell lines, metastatic melanoma cell lines generally showed a lack of correlation between DNA amplification and gene expression. While in metastatic cell lines copy number gains were frequently detected in *MHC II-α*, *MHC-IIβ*, *MHC-Iα*, *TAP*, and *LMP* (all located on chromosome 6) (Supplemental Fig. 2), multiple *MHC-Iα* genes were in fact significantly down-regulated (Fig. 2B and Table 2). Other genes showed similar expression between metastatic melanoma lines and melanocytes. *HLA-DRB1* and *CD74* were the only two genes still overexpressed, but the enhancement in expression (~2 folds) was far less compared to the enhancement in VGP lines (~14 fold). Thus, all components of the antigen-presenting pathway were significantly down-regulated in metastatic lines when compared to VGP. This relationship can be observed in the Ingenuity software (Fig. 3 and Table 3). These not only include the *MHC* genes on chromosome 6, but also the *MHC-Iβ* gene *B2M* on chromosome 15 and *CD74* on chromosome 5. In fact, unbiased Ingenuity analysis of the significantly altered pathways between metastatic and VGP cell lines identified the most significant pathway as the antigen presentation pathway ($P=9.54 \times 10^{-14}$). To validate the expression changes observed with microarray, PCR-based gene expression assays were performed on two *MHC* molecules, *CD74* (*HLA-DG*) and *HLA-DRA*. Both *CD74* and *HLA-DRA* were not detectable in melanocytes, but were expressed at high levels in VGP cell lines (Fig. 4). Notably, in VGP lines, when comparing to DNA amplification, for all the cell lines that showed *HLA-DRA* gene amplification, overexpression was also observed (Table 4). However, for certain VGP cell lines with no DNA amplification, overexpression was also observed, suggesting DNA amplification could be one of the mechanisms contributing to the overexpression of the *MHC* molecules in VGP lines. In metastatic cell lines, the expression of *CD74* and *HLA-DRA* was significantly reduced comparing to VGP lines (Fig. 4). Some metastatic cell lines did show certain degree of low level expression, while none of the melanocytes had any detectable level of *CD74* or *HLA-DRA* mRNA expression by taqman. This was further confirmed with Western blot analysis for protein level detection of the same two molecules in a subset of the cell lines with RNA data (Fig. 4, top portion). The control *GAPDH* level was relatively constant among the cell lines. Together, these results revealed a shift of expression pattern of the *HLA* genes as melanoma cells progress from the VGP to the stage of metastasis.

Down-Regulation of *CIITA* in Metastatic Melanoma Cell Lines

CIITA is an important transactivator of *MHC-II* genes. In order to investigate the possible mechanism of decreased *MHC-II* expression in metastatic melanoma cell lines, we assessed the mRNA expression of *CIITA* by using RT-taqman. *CIITA* was not expressed in melanocytes, but it was highly expressed in VGP cell lines. In contrast, the majority of metastatic cell lines showed undetectable or low level of *CIITA* expression (Fig. 5). This down-regulation of *CIITA* from VGP to metastasis may be responsible for the reduced expression of *MHC-II* in the metastatic cell lines.

DISCUSSION

Although *MHC* molecule abnormalities are frequently found in melanoma cells, their stage-specific expression has not been well documented. The results presented here demonstrate that the expression of *MHC* molecules in melanomas vary greatly by tumor stage. When analyzed separately, VGP and metastatic melanoma demonstrate unique patterns of *MHC* expression despite similar trends in DNA copy number. Specifically, multiple *MHC-I* and *MHC-II* genes were overexpressed in VGP melanoma but not in metastatic melanomas.

MHC-I expression was reported to be down-regulated in melanoma (Ferrone and Marincola, 1995) as well as in melanoma cell lines (Mendez et al., 2008). Here we discovered that this down-regulation is tumor stage specific. Multiple *MHC-I* molecules were significantly down-regulated in metastatic melanoma cell lines relative to melanocytes (Table 2), consistent with previous reports. However for the VGP lines, the down-regulation was rare (Table 1). In fact, multiple *MHC-I* molecules were expressed at significantly higher level in VGP lines than in melanocytes. Comparison of metastatic cell lines with VGP lines showed overall lower expression of *MHC-I* molecules in metastatic cell lines (Table 3). A cautionary note is that this is a genomic study examined changes at the mRNA level, while most of the earlier studies were using antibodies to detect protein expression on cell membrane. It is possible that *MHC-I* complexes could be further down-regulated via other mechanisms mentioned previously (Mendez et al., 2008). By using a microarray platform we were able to look at the overall picture of all the *MHC* molecules, while protein studies are restricted to one molecule at a time and are limited by antibody availability and specificity, among other characteristics. At least at mRNA level, there seem to be significant less *MHC-I* expression in metastatic cell lines comparing to VGP lines. HLA class I alteration was reported in 67% of the ESTDAB melanoma cell lines (Mendez et al., 2008). It would be interesting to find out if the cell lines that did not show alteration are enriched with VGP lines.

Constitutive expression of *MHC-II* molecules is usually restricted to the cells of the immune system and thymic epithelial cells (Mach et al., 1996). However, different tumors have been reported to express *MHC-II* molecules (Altomonte et al., 2003). Here we identified that the VGP melanomas were the predominant type overexpressing the *MHC-II* molecules. There was a significant down-regulation of the *MHC-II* expression in metastatic cell lines relative to the VGP lines (Table 3). In the ESTDAB collection of melanoma cell lines, it was also noted that *MHC-II* expression was only observed in a portion of the cell lines, while 24 out of 42 cell lines had no constitutive or IFN- γ induced expression. In a different study on VGP and lymph node metastases, though consistent with our result that VGP tumors expressed *MHC-II* molecules, *MHC-II* expression was also reported in a subset of lymph node metastases and was found to correlate with better survival (Anichini et al., 2006). It has been reported that melanomas isolated at different body sites, with different UV exposure etc. can have distinct sets of genetic alterations and consequently different expression profiles (Curtin et al., 2005). Additionally, the expression of HLA molecules is greatly influenced by IFN- γ . All the metastatic cells in the article by Anichini et al. (2006) were isolated from lymph nodes and were only grown in short culture which might play a role in more *MHC* class II molecule expression as well. We did notice that in some metastatic cell lines, like WM1361B, there was expression of HLA-DRA, but at a low level (Table 4 and Fig. 4). It would not be very surprising if the expression level were increased dramatically upon IFN- γ treatment.

The shift in gene expression pattern in metastatic melanomas from that seen in VGP, with significantly reduced expression of multiple *MHC* class I and II genes in metastatic melanomas, indicates a mechanism of tumor cell immune evasion and supports the cancer immune surveillance theory. The change of *MHC* expression during progression from VGP to metastasis also suggests different roles of *MHC* molecules in different stages of melanoma. *MHC* molecules may function in promoting tumor progression and local tumor-specific immune suppression in non-metastasis stages, such as the VGP stage, possibly via mechanisms mentioned previously (Aoudjit et al., 2004; Wang et al., 2005). Conversely, in metastatic melanoma cells, eliminating *MHC-II* expression in conjunction with down-regulation of *MHC-I* may further enhance immune evasion of melanoma cells. This may facilitate the process of metastasis that includes invasion, intravasation, extravasation, and the growth of secondary tumor.

In this study we also observed that the overexpression of the *MHC* molecules in VGP cells were associated with copy number gains at the *MHC* complex loci on chromosome arm 6p. The fact that multiple genes located in the same genomic loci on chromosome 6 exhibited increase in mRNA level, and this increase was not observed for genes not on chromosome 6 like the *MHC*-I β molecules, suggests that DNA amplification contributed to the overexpression of the *MHC* molecules in VGP. We did notice that for all the VGP lines that had amplification in the *MHC* loci, HLA-DRA expression was upregulated. But for some VGP lines that did not show DNA amplification, overexpression was also observed (Table 4). This is often seen in the cases of oncogene amplification and overexpression, in that overexpression exceeds amplification, suggesting that DNA amplification is just one of the mechanisms to upregulate the expression of a gene that is needed for cancer progression (Prins et al., 1993; Hynes and MacDonald, 2009; Kim and Salgia, 2009). Despite the frequent occurrence of DNA copy number gains also in metastatic cells, *MHC* molecule expression level was significantly lower than VGP. While copy number gains in VGP and metastatic melanomas at the *MHC* loci could contribute to the overexpression of the *MHC* genes, metastatic melanomas may harbor additional mechanisms involved in regulating the expression of *MHC* molecules that “override” the effect of copy number gain and lead to decrease of gene expression.

Interestingly, studies on different mechanisms that could lead to loss of *MHC*-I expression in the ESTDAB melanoma cell line collection revealed LOH of the *MHC* loci on chromosome arm 6p as one of the potential mechanisms (Rodriguez et al., 2005; Mendez et al., 2008). The region discussed in this paper 6p21 is one of the regions frequently found to be amplified in human cancers including melanoma (Santos et al., 2007). It appeared not to be a cell line artifact since we observed this amplification in both cell lines as well as melanoma tumors. 6p amplification was also reported in other studies on primary and metastatic melanomas (Balazs et al., 2001). The most compelling evidence came from a report on 126 melanomas where 6p amplification was reported to be a frequent event in melanoma (Curtin et al., 2005). There could be multiple reasons for the discrepancy. One possibility is different tissue collection; melanomas isolated at different body sites, with different UV exposure etc. can have distinct sets of genetic alterations (Curtin et al., 2005). Another possibility is different methods; most of the 6p LOH reports were done using microsatellite marker amplification while 6p amplification was detected using CGH method to reflect dosage change. As reported in this paper, most of the 6p copy number gains are low level gains of less than 5 copies. It is possible that one allele can be lost and the other allele is amplified and the net result is still the gain of dosage. We did notice that for the ESTDAB collection, even though 6p LOH was reported based on microsatellite marker analysis, the FISH result always showed at least two hybridization signals, and often 3 or 4 signals (Mendez et al., 2008), consistent with our observation of low level DNA amplification at 6p. The fact that for most of the cell lines in the ESTDAB collection down-regulation of HLA locus could be reversed after treatment with IFN- γ makes it highly unlikely that loss occurred at a DNA level.

Due to reports indicating frequent constitutive expression of *MHC*-II molecules in melanoma cells were caused by abnormal constitutive transcription of *CIITA* (Deffrennes et al., 2001; Goodwin et al., 2001), the master regulator of *MHC*-II gene expression, we explored the expression of *CIITA* as a potential mechanism of *MHC*-II down-regulation in metastatic melanoma cells. We note the same pattern of *CIITA* expression as the expression of *MHC*-II molecules, using CD74 and HLA-DRA as examples. *CIITA* expression was high in VGP cell lines but decreased dramatically in metastatic cell lines (Fig. 5), suggesting that reduced *CIITA* level may be responsible for down-regulation of *MHC*-II in metastatic melanoma cells relative to VGP lines. Thus, despite DNA copy number gains, *MHC*-II expression in metastatic melanoma is possibly controlled by the transcriptional activity of

CIITA. Possible mechanisms of *CIITA* expression reduction include promoter methylation, deacetylation of histone, or methylation of Histone H3 lysine 9 (van den Elsen et al., 2004; Holling et al., 2006; Wright and Ting, 2006). We also observed down regulation of *MHC-I* molecules in metastatic melanoma. The potential mechanisms for *MHC-I* underexpression can be epigenetic silencing or regulation by transcriptional factors (van den Elsen et al., 2003). Interestingly, it was also shown that *CIITA* has a role in *MHC-I* expression (Martin et al., 1997), suggesting potential contribution of *CIITA* to the down-regulation of *MHC-I* in metastatic melanoma.

In conclusion, this study explored *MHC* class molecule expression in different stages of melanoma. We have revealed a distinct pattern of *MHC* expression with tumor stage despite similar DNA copy number changes. Further, we identified potential causes of this shift in gene expression pattern. Since immunologic events play such important roles in the clinical course of melanoma, besides serving as molecular markers of melanoma progression, the distinct expression pattern of *MHC* molecules in metastatic melanoma vs. VGP can also have important implications in immunological interventions against the disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Copy number aberrations of *MHC* molecules in melanoma cells. Copy number gains of *MHC*-I and *MHC*-II (6p21.32) are common in melanomas. Vertical green bars represent the extent of DNA copy number gains along chromosome 6 in a panel of 52 cancer cell lines (A) and 43 tumor tissues (B). With respect to the copy number changes, the cell lines are good models for the tumor tissues, where the copy number gain frequency is 32.7% and 25.6% respectively. Only 3 samples (1 cell line, 2 tumors) harbored DNA gains of > 5 copies (dark green).

Figure 2.

Aberrant expression of *MHC* molecules in VGP melanoma cell lines and metastatic melanoma cell lines compared to melanocytes. (A) Aberrant expression of *MHC* molecules in the antigen presentation pathway in VGP melanoma cells compared to melanocytes viewed with the Ingenuity pathway analysis software. Significantly differentially expressed genes (P -value < 0.05) with fold change over 1.5 between VGP cells and melanocytes were loaded into and viewed with the Ingenuity pathway analysis software. Green represents genes with increased expression and red represents genes with decreased expression compared to melanocytes. (B) Aberrant expression of *MHC* molecules in the antigen presentation pathway in metastatic melanoma cells compared to melanocytes viewed with the Ingenuity pathway analysis software. Significantly differentially expressed genes (P -value < 0.05) with fold change over 1.5 between metastatic cells and melanocytes were loaded into and viewed with the Ingenuity pathway analysis software. Green represents genes with increased expression and red represents genes with decreased expression compared to melanocytes.

Figure 3.

Significant decrease of gene expression in the antigen presentation pathway in metastatic melanoma cell lines in comparison to VGP melanoma cell lines. Significantly differentially expressed genes (P -value < 0.05) between metastatic and VGP melanoma cell lines, also with fold change over 1.5, were loaded into and viewed with the Ingenuity pathway analysis software. Red represents decreased expression of components of this pathway in metastatic cells compared to VGP cells.

Figure 4.

Taqman gene expression assay and Western blot show that expression levels for HLA-DRA and CD74 are non-detectable in melanocytes and very low in metastatic cell lines, but are strongly elevated in VGP melanoma cell lines. HLA-DRA (A) and CD74 (B) mRNA levels were measured by rt-taqman in a group of melanocyte cell lines, VGP lines and metastatic melanoma cell lines, and normalized to β -actin house-keeping gene mRNA level. The result presented here is the average of two independent data points from a single experiment. The same experiment was done twice with similar results. The names of the cell lines in the Figures are listed in Table 4 in the same order. The protein extracts were made from a subset of these cell lines and probed for HLA-DRA and CD74 protein levels by western. GAPDH was used as loading control.

Figure 5.

Taqman gene expression assay shows that in concordance with HLA-DRA expression, *CIITA* is highly expressed in VGP cell lines, but is non-detectable in melanocytes and expressed at very low level in metastatic cell lines. HLA-DRA and *CIITA* mRNA levels were measured by rt-taqman, and relative expressions to β -actin were plotted side-by-side for the same cell lines. The result presented here is the average of two independent data points from a single experiment. The same experiment was done twice with similar results. The names of the cell lines in the Figure are listed in Table 4 in the same order.

TABLE 1

MHC Genes Whose Expression is Significantly Altered in VGP Melanoma Cells Relative to Melanocytes ($P < 0.05$).

Probe	Gene	Adjusted <i>P</i> -value	Fold change
210982_s_at	HLA-DRA	0.006	16.50
208894_at	HLA-DRA	0.003	15.16
209312_x_at	HLA-DRB1	0.009	14.22
209619_at	CD74	0.002	13.70
215193_x_at	HLA-DRB4	0.009	11.81
204670_x_at	HLA-DRB5	0.01	6.09
211990_at	HLA-DPA1	0.026	5.57
208306_x_at	HLA-DRB4	0.005	4.95
211991_s_at	HLA-DPA1	0.02	4.76
212671_s_at	HLA-DQA1	0.049	4.58
217478_s_at	HLA-DMA	0.018	4.12
209823_x_at	HLA-DQB1	0.031	3.29
211656_x_at	HLA-DQB1	0.03	2.98
203932_at	HLA-DMB	0.002	2.84
211654_x_at	HLA-DQB1	0.012	2.48
201137_s_at	HLA-DPB1	0.036	2.48
204279_at	LMP2	0.014	2.22
209140_x_at	HLA-B	0.023	2.21
217362_x_at	HLA-DRB1	0.017	2.03
208812_x_at	HLA-C	0.05	1.75
204769_s_at	TAP2	0.047	1.57
209040_s_at	LMP7	0.039	1.52
213932_x_at	HLA-A	0.047	0.74
222279_at	HLA-F	0.001	0.42

Genes are listed in the order of fold change between VGP melanoma cells and melanocytes.

TABLE 2

MHC Genes Whose Expression is Significantly Altered in Metastatic Melanoma Cells Relative to Melanocytes ($P < 0.05$).

Probe	Gene	Adjusted <i>P</i> -value	Fold change
215193_x_at	HLA-DRB1	0.0230	2.21
209619_at	CD74	0.0390	1.95
217001_x_at	HLA-DOA	0.0130	0.75
211530_x_at	HLA-G	0.0280	0.67
211529_x_at	HLA-G	0.0160	0.66
217456_x_at	HLA-E	0.0140	0.64
217436_x_at	HLA-J	0.0060	0.64
210514_x_at	HLA-G	0.0100	0.63
211528_x_at	HLA-G	0.0030	0.62
221875_x_at	HLA-F	0.0040	0.61
204806_x_at	HLA-F	0.0050	0.58
200905_x_at	HLA-E	0.0030	0.53
215313_x_at	HLA-A	0.0020	0.52
200904_at	HLA-E	0.0080	0.44

Genes are listed in the order of fold change between metastatic melanoma cells and melanocytes.

TABLE 3

MHC Genes Whose Expression is Significantly Altered in Metastatic Melanoma Cells Relative to VGP Melanoma Cells ($P < 0.05$).

Probe	Gene	Adjusted <i>P</i> -value	Fold change
216231_s_at	B2M	0.0230	0.77
211530_x_at	HLA-G	0.0060	0.76
210514_x_at	HLA-G	0.0230	0.75
217456_x_at	HLA-E	0.0440	0.74
211529_x_at	HLA-G	0.0400	0.73
211528_x_at	HLA-G	0.0230	0.69
204806_x_at	HLA-F	0.0220	0.69
202307_s_at	TAP1	0.0430	0.67
214459_x_at	HLA-C	0.0170	0.61
216526_x_at	HLA-C	0.0200	0.61
208812_x_at	HLA-C	0.0250	0.58
211911_x_at	HLA-B	0.0490	0.58
208729_x_at	HLA-B	0.0230	0.57
204279_at	LMP2	0.0370	0.57
211799_x_at	HLA-C	0.0030	0.55
209140_x_at	HLA-B	0.0410	0.55
200904_at	HLA-E	0.0260	0.55
203932_at	HLA-DMB	0.0150	0.53
217362_x_at	HLA-DRB6	0.0070	0.51
211654_x_at	HLA-DQB1	0.0200	0.46
213537_at	HLA-DPA1	0.0310	0.42
211656_x_at	HLA-DQB1	0.0210	0.38
209823_x_at	HLA-DQB1	0.0290	0.38
212998_x_at	HLA-DQB1	0.0480	0.36
201137_s_at	HLA-DPB1	0.0040	0.33
212671_s_at	HLA-DQA1	0.0410	0.32
217478_s_at	HLA-DMA	0.0050	0.27
208306_x_at	HLA-DRB4	0.0020	0.26
204670_x_at	HLA-DRB5	0.0010	0.26
211991_s_at	HLA-DPA1	0.0040	0.23
211990_at	HLA-DPA1	0.0090	0.21
215193_x_at	HLA-DRB1	0.0040	0.17
209312_x_at	HLA-DRB1	0.0020	0.16
209619_at	CD74	0.0010	0.12
208894_at	HLA-DRA	0.0020	0.10
210982_s_at	HLA-DRA	0.0020	0.10

Genes are listed in the order of fold change between metastatic melanoma cells and VGP melanoma cells.

TABLE 4

The DNA Copy Number and mRNA Expression Status of HLA-DRA in Melanoma Cell lines and Melanocytes

Order	Cell line	Lesion Type	HLA-DRA copy number	HLA-DRA relative expression
1	FOM101-1	Mel		0.00
2	FOM103-1	Mel		0.00
3	FOM113-1	Mel		0.00
4	FOM92-1	Mel		0.00
5	FOM92-1 (2) (P3)	Mel		0.00
6	FOM92-1 (P10)	Mel		0.00
7	FOM99-1	Mel		0.00
8	WM115	VGP	No Change	8.13
9	WM1346	VGP	N/A	0.22
10	WM1361A	VGP	No Change	0.38
11	WM1366	VGP	Gain < 5 copies	0.13
12	WM1819	VGP	N/A	0.00
13	WM278	VGP	Gain < 5 copies	4.31
14	WM3248	VGP	Gain < 5 copies	7.38
15	WM3268	VGP	Loss 1 copy	2.60
16	WM39	VGP	No Change	0.00
17	WM75	VGP	No Change	15.27
18	WM793b	VGP	No Change	0.59
19	WM902b	VGP	Gain < 5 copies	2.78
20	WM983A	VGP	N/A	0.00
21	WM1205lu	Met	N/A	0.02
22	WM1361B	Met	N/A	0.91
23	WM1361C	Met	N/A	0.45
24	WM1382	Met	No Change	0.00
25	WM1617	Met	N/A	0.00
26	WM164	Met	No Change	0.00
27	WM1727A	Met	No Change	0.00
28	WM239A	Met	N/A	0.01
29	WM266-1	Met	N/A	0.24
30	WM266-2	Met	N/A	0.27
31	WM3451	Met	N/A	0.88
32	WM46	Met	No Change	0.04
33	WM51	Met	No Change	0.03
34	WM8	Met	Gain < 5 copies	0.00
35	WM852	Met	N/A	0.28
36	WM858	Met	No Change	0.00
37	WM873-2	Met	N/A	0.01
38	WM88	Met	N/A	0.32

Order	Cell line	Lesion Type	HLA-DRA copy number	HLA-DRA relative expression
39	WM983B	Met	N/A	0.00
40	WM983C	Met	Gain < 5 copies	0.02

The DNA copy number status is based on aCGH data; the mRNA expression is listed as relative expression to β -actin measured by rt-taqman. The cell lines are listed in the same order as in Figs 4 and 5.