Nucleic Acids Research, 1993, Vol. 21, No. 5 1179–1185

Downregulation of HLA Class I expression by c-myc in human melanoma is independent of enhancer A

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Received December 4, 1992; Revised and Accepted February 8, 1993

ABSTRACT

High constitutive expression of the c-myc oncogene in human melanoma leads to downregulation of expression of HLA Class I genes. The genes at the HLA-B locus are preferentially affected. To investigate the mechanism of downregulation, the activity of the main HLA Class I enhancer, enhancer A-region I, was compared in a panel of c-myc transfectants with increasing myc expression. Gel retardation experiments demonstrated in all tested cell lines binding of the transcription factors KBF1 and NF-κB to the enhancer. However, no correlation between the levels of HLA Class I expression and binding to the enhancer could be established. Strikingly, the cell line with the highest c-myc expression showed more binding of KBF1 and NF-κB than the parental cell line. By using CAT reporter plasmids in transient transfection assays we investigated the *in vivo* function of enhancer A-region I in the c-myc transfectant panel. Again, c-myc expression had no effect at all on the activity of enhancer A. This study shows that HLA Class I expression is regulated by the c-myc oncogene at the level of transcription, but that the main HLA Class I enhancer is not involved in this process.

INTRODUCTION

Genes of the Major Histocompatibility Complex (MHC, HLA in humans, H-2 in mice) encode proteins that are crucial for recognition of foreign proteins by the immune system of the vertebrate. The classical HLA Class I antigens are polymorphic proteins encoded by three different loci (A, B and C) and are expressed at the surface of all somatic cells. Presentation of intracellular antigens to immune effector cells is effectuated by binding of endogenously produced protein fragments to the peptide-binding groove of HLA molecules formed by their α1 and α2 domains. After non-covalent binding of the HLA/peptide complex to β_2-microglobulin, the ternary complex reaches the cell surface (1). Modulation of expression of MHC Class I proteins is frequently found in tumor cells of different origin (2,3) and may lead to alteration of the immune response to these cells. A link between the action of oncogenes and modulation of the immune defense was implied by the finding that nuclear oncogenes such as c-myc (4,5), N-myc (6) and the adenovirus 12 E1A oncogene (Ad12 E1A) (7,8) are capable of reducing the MHC Class I mRNA level. Since MHC Class I products have such a important function in determining the elimination of tumor cells by either T cells (9) or NK cells (10), it is important to understand how the regulation of MHC Class I genes in tumor cells can be affected.

The promoter region of MHC Class I genes has been studied in great detail (11,12) and was found to contain the general CAAT and TATA boxes and two *cis*-acting regulatory elements, termed enhancer A and enhancer B, which are located approximately 150 and 80 bp upstream of the transcription start site, respectively. In various cell types enhancer A appears to be the prominent activator of Class I transcription by its capability to bind several *trans*-acting factors (13–17). Of these, the ubiquitous factors H2TF1 and KBF1 and another transcription factor, NF-κB, share an identical DNA binding subunit, which is called p50 (18). The core sequence of enhancer A, termed region I, is highly conserved in H-2 and HLA Class I genes. In addition, region I shows homology with an element found in the β_2-microglobulin gene and in some NF-κB-regulated genes (19). In many cases where the mechanism of MHC Class I reduction has been studied, the function of this enhancer was found to be affected. Studies on MHC Class I-negative tumor cells have revealed an alteration of enhancer A function (19–21) and also in systems where the effector gene is known, *i.e.* N-myc or Ad12 E1A, inactivation of this enhancer was reported (22–24).

We have previously shown that overexpression of the c-myc oncogene in human melanoma leads to a reduction of HLA Class I expression (4) and that the c-Myc protein mainly affects the level of expression of HLA-B locus products (5). Locus-specific loss of MHC Class I expression in tumors has also been described for other tumors (reviewed in (3)) and was in the case of colorectal carcinoma correlated with alteration of binding activity to the enhancer A region (21). To scrutinize the role of enhancer A in the locus-specific downregulation of HLA Class I expression by the c-myc oncogene, we explored the activity of this enhancer in a panel of c-myc transfectants *in vitro* and *in vivo*. Because expression of N-myc, which is highly

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homologous to c-myc, was known to affect specifically region I in enhancer A, we focused our research on the activity of this element. We demonstrate that, unexpectedly, c-myc has no effect on this element.

MATERIALS AND METHODS

Northern and Western analysis

Isolation of total RNA, Northern blotting, preparation and radiolabeling of the probes was carried out as described before (5). The cDNA of human elongation factor (HEF) (25) was used as a control probe. The human c-myc, the rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (4) and HEF probes were hybridized at 42°C, the HLA-A and HLA-B probes (5) were hybridized at resp. 59.5°C and 62.5°C. In the latter two cases, the filters were washed three times for 5 minutes in 2×SSC, 0.1% SDS at 50°C. Western blotting was performed as has been described previously (26).

Tissue culture and transfections

Melanoma cell lines and mouse L cells (5) were cultured in DMEM (Gibco, Paisley, Scotland) supplemented with 8% FCS. The generation of the c-myc transfectants has been described before (4). Transient transfections were performed by the calcium-phosphate precipitation method (27). Cells were seeded in 100 mm plates one day before transfection. The melanoma cells were transfected at 50−70% density with 3 μg of luciferase plasmid or 10 μg of CAT construct, together with 5 μg of a β-galactosidase expressing control plasmid (pCMV-gal). Cells were harvested 48 hr post transfection.

Plasmids

For construction of the HLA-B7 luciferase plasmid (pB7−653/+13luc) we first inserted the 666 bp *Eco*RI-*Aha*II fragment of the HLA-B7 promoter into the *Xba*I site of pBLCAT5 (28). The CAT gene was subsequently replaced by an *Xho*I-*Hpa*I luciferase fragment from pGL2-Basic (Promega, Madison, WI). MHC-conaCAT and mhc-conaCAT were constructed by ligation of the oligomers 5′-GCCCAGGGCTGGGGATTCCCCA and 5′-GCCCAGGGCTGCGGATTCCCGA, respectively, into the *Bam*HI site of the conalbumin-CAT plasmid (11,24). The β-galactosidase control vector pCMV-gal was constructed by inserting a fragment containing the cytomegalovirus (CMV) immediate early enhancer/promoter upstream of the β-galactosidase gene in pCH110 (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Luciferase assays

Cells were harvested 48 hr post transfection by scraping in PBS. The cell pellet was resuspended in Cell Culture Lysis Reagent (Promega, Madison, WI). After 15 minutes incubation at room temperature the cell debris was removed by brief centrifugation. The luciferase activity in a 20 μl sample of the supernatant was determined by adding 100 μl of Luciferase Assay Reagent (Promega, Madison, WI), followed by 10 seconds of vortexing. Immediately, the produced light was measured for 10 seconds in a luminometer (Tropix, Bedford, MA). To correct for the transfection efficiency, the β-galactosidase activity in the extracts was determined by using 3-(4-Methoxyspiro[1,2-dioxetane-3, 2′-tricyclo[3.3.1.1^{3,7}]decan]-yl)phenyl β-D-galactopyranoside (AMPGD, Tropix, Bedford, MA) as a chemiluminescent substrate according to the manufacturer. The relative luciferase activity of the samples is represented as the ratio of the produced light units of the respective luciferase and β-galactosidase analyses.

CAT assays

For CAT reporter assays the cells were harvested by treatment with PBS, 2mM EDTA followed by 3 freeze and thaw cycles (29). CAT enzyme activity was determined according to reference (30) after inactivation of the extract for 10 minutes at 65°C. The butyrylated product was isolated by one xylene extraction and one back-extraction with TE. The amount of catalytic units present in the cell extracts was determined using a standard curve of purified CAT enzyme (Promega, Madison, WI). β-Galactosidase activity in the extracts was assayed in 96 wells plates using o-nitrophenyl-β-D-galactopyranoside (ONPG) as substrate (29). The amount of catalytic units present in the cell extracts was estimated according to a standard curve of purified β-galactosidase (Promega, Madison, WI). The relative CAT activity in the cell extracts is represented as the ratio of CAT units and β-galactosidase units.

Protein extracts and gel retardation assays

Preparations of whole cell extracts by high salt extraction, binding reactions and electrophoresis of the complexes were carried out according to reference (31), except that binding buffer without GTP was used. The probes tested were: the wild-type MHC probe (5′-CGGCTGGGGATTCCCCATCT-3′) and the mutant (mhc) probe (5′-CGGCTGCGGATTCCACATCT-3′) as a control. Both probes have been cloned into the *Bam*HI site of pUC13. Probes were released by digestion with *Eco*RI and *Hin*dIII and labeled with Klenow enzyme in the presence of 30 μCi of [α-^{32}P]dCTP (3000 Ci/mmol). The 75 bp fragments were separated from the vector by electrophoresis on a native 6% polyacrylamide gel, followed by electro-elution in 0.05 × TBE. When polyclonal antisera were tested, the whole cell extracts were incubated for 15 minutes in buffer supplied with 1 μl of the appropriate antiserum. The labeled probe was added, followed by incubation for 30 minutes.

RESULTS

Locus-specific downmodulation of HLA-B by c-myc

The locus-specific downmodulation of HLA-B products by c-myc overexpression was previously discovered by studying a panel of melanoma cell lines and two series of c-myc transfectants (4,5). To unravel the mechanism of this downregulation we made use of one of these panels, namely cell line IGR39D and its c-myc-transfected clones. A Northern blot of total RNA of the independent IGR-myc clones hybridized with a c-myc probe shows very high levels of transfected c-myc mRNA in clone 7 and 3, whereas clone 6 has a lower expression (Fig. 1A). This result is in accordance with the amount of c-Myc protein present in these clones as determined by Western blotting (Fig. 1B). Only in the transfectants clone 7 and 3 Myc protein can be detected by this method (Fig. 1B, lanes 3 and 4), indicating that in clone 6 the c-Myc protein expression is below the level of immunodetection. Locus-specific hybridization of the Northern blot with an HLA-B8 probe shows in c-myc-transfected clones 7 and 3 strongly reduced amounts of HLA-B mRNA as compared with the parental cell line IGR39D and clone 6 (Fig. 1A). In contrast, hybridization with a locus-specific HLA-A probe demonstrates that the level of HLA-A mRNA expression remains

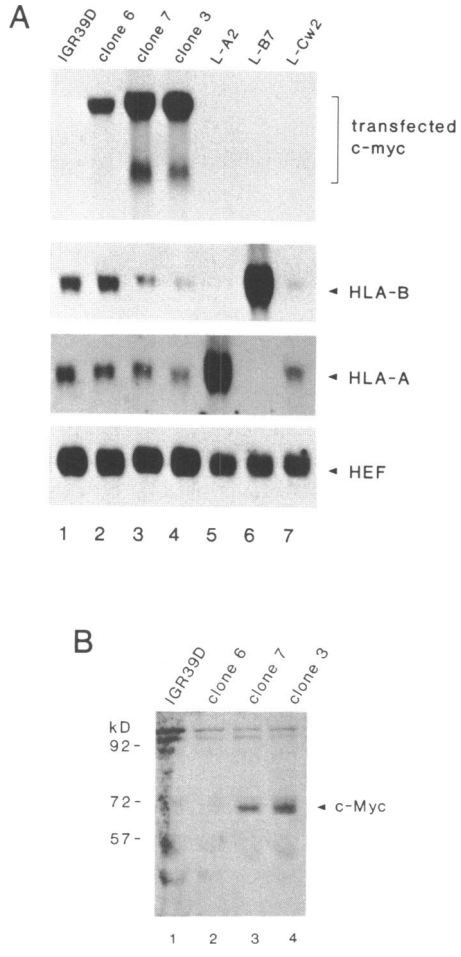

Figure 1. HLA Class I and c-myc expression levels in melanoma transfectants. **(A)** Northern analysis of RNA isolated from melanoma cell line IGR39D (lane 1), its c-myc transfectants clone 6, 7 and 3 (lanes 2–4) and mouse L cells expressing transfected Class I HLA-A2, -B7 and -Cw2 genes, respectively (lanes 5–7). The filter was sequentially hybridized with HLA-B, HLA-A, c-myc and human elongation factor (HEF) probes. **(B)** Western blot analysis of whole cell protein extracts from IGR39D (lane 1) and its c-myc transfectants clone 6, 7 and 3 (lanes 2–4) stained with anti-Myc monoclonal antibody 9E10 (40).

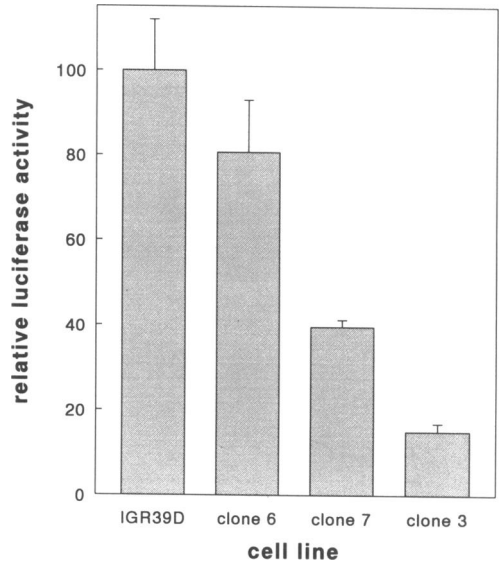

Figure 2. Transcriptional activity of the HLA-B promoter region in c-myc-transfectants. Cell line IGR39D and its c-myc transfectants clone 6, 7 and 3 (as indicated) were transiently transfected with a construct comprising the luciferase reporter gene under the control of the HLA-B7 promoter region (−653/+13). The relative luciferase activity, as corrected for transfection efficiency by β-galactosidase coexpression is expressed as percentage of the activity in IGR39D. The values given are mean values of two representative transfection experiments, each performed in triplicate.

essentially unaltered by the c-myc overexpression (Fig. 1A). This shows that the level of expression of HLA-B in this panel is inversely correlated with c-myc expression, whereas HLA-A mRNA levels are much less affected.

HLA Class I expression influenced at the transcriptional level

In many systems it has been shown that regulation of expression of MHC Class I genes takes place at the transcriptional level (32). Therefore, we asked whether also in the case of downmodulation by c-myc in melanoma cells the transcriptional activity of the HLA-B promoter region is affected. To investigate this, a luciferase reporter construct comprising a 666 bp proximal HLA-B7 promoter fragment was transiently transfected in the three above described IGR-myc clones and their parental cell line. The transcriptional activity of the plasmid was determined in four independent transfection experiments and corrected for differences in transfection efficiencies by β-galactosidase co-expression. The mean values obtained from two representative experiments, each consisting of three independent precipitates, were expressed as relative luciferase activities and compared with the parental cell line IGR39D. As can be seen in Figure 2, the luciferase activity of the HLA-B7 promoter construct is reduced in the three IGR-myc transfectants. This reduction is most significant in the myc-clones with the highest c-myc expression, clone 7 and clone 3, in agreement with the low HLA-B mRNA levels in these clones. This indicates that c-myc is capable of regulating the expression of HLA-B genes by affecting their transcription rate and that the promoter region of 0.7 kb upstream of the cap site is involved in the regulation.

In vitro binding activity to enhancer A not correlated with c-myc expression

Reports on the c-myc-related oncogenes N-myc and Ad12 E1A, which also mediate downregulation of MHC Class I, indicated transcriptional regulation of the genes by inactivation of enhancer A (22–24). Its core sequence, frequently called the region I element, is remarkably conserved in many H-2 and HLA Class I alleles (Fig. 3). Only the sequences of HLA-Aw24, the HLA-C genes and H-2D^p are distinct from the consensus. To determine whether the binding of proteins to this enhancer is affected by c-myc, gel retardation assays with whole cell extracts of the c-myc transfectants and four other melanoma cell lines differing in their HLA-B expression (5) were performed. As probes we used the MHC enhancer and its mutant version that is unable to bind the transcription factors KBF1, H2TF1 and NF-κB. To exclude any possible involvement of variation between different isolations, at least three independent extract preparations of the used cell lines were tested and the results were proven to be reproducible. A representative experiment is shown in Figure 4. In all melanoma cell lines and transfectants we observed two different bands specific for the wild-type probe (Fig. 4A and

Gene	Sequence
HLA-A1	$^{-155}$ TGGGGATTCCCCA^{-144}
HLA-A2	------------
HLA-A3	------------
HLA-A11	------------
HLA-Aw24	----•-------•-
HLA-B7	------------
HLA-B27	------------
HLA-B35	------------
HLA-B51	------------
HLA-B57	------------
HLA-Cw1	--A------T---
HLA-Cw2	--A------T---
HLA-Cw5	--A------T---
H-2D^b	------------
H-2D^k	------------
H-2D^p	----------AT
H-2K^b	------------
H-2K^k	------------
H-2L^d	------------

Figure 3. Sequences of enhancer A-region I in MHC Class I genes. The consensus sequence is given in the first line, the nucleotides diverging from the consensus sequence are indicated or when absent, represented as a dot. The HLA-B7 and HLA-B51 sequences were communicated by E.H.Weiss, H-2K^k sequence was communicated by R.S.Goodenow, the other sequences were taken from the literature: HLA-A1 (41), HLA-A2 (42), HLA-A3 (43), HLA-A11 (44), HLA-Aw24 (45), HLA-B27 (46), HLA-B35 (47), HLA-B57 (48), HLA-Cw1 and HLA-Cw2 (49), HLA-Cw5 (50), H-2D^b and H-2D^k (51), H-2D^p (52), H-2K^b (11) and H-2L^d (53).

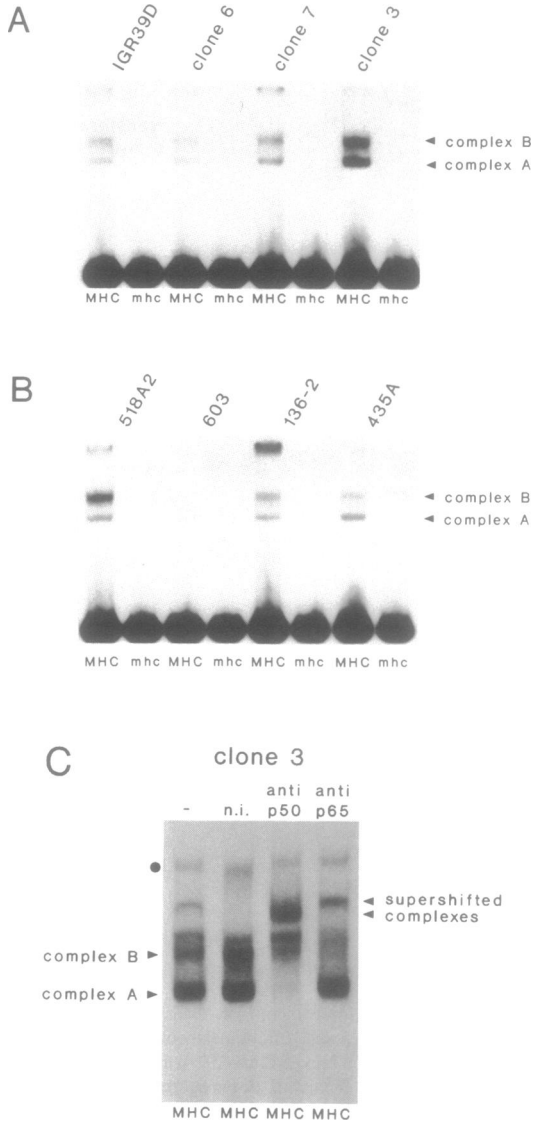

Figure 4. Gel retardation analysis of the MHC enhancer with protein extracts of melanoma cell lines and transfectants. As probes, the MHC enhancer (MHC) or the mutated MHC element (mhc) were used. **(A)** Retardation assay with whole cell protein extracts of IGR39D and its c-myc transfectants clone 6, 7 and 3, as indicated. **(B)** Retardation assay with whole cell protein extracts of melanoma cell lines 518A2, 603, 136-2 and 453A, as indicated. **(C)** Retardation assay with whole cell extract of c-myc transfectant clone 3. Cell extracts were preincubated with polyclonal antisera against p50 (lane 3) and p65 (lane 4), or rabbit non-immune serum (lane 2).

B, complex A and B in lanes 'MHC'). From the experiments shown in Figure 4A, it can be noticed that the amounts of protein binding to the wild type probe in IGR39D and the c-myc transfectants show neither a correlation with HLA Class I expression nor an inverse correlation with c-myc expression. Rather, the intensities of the retarded bands in clone 3, which has the lowest HLA-B expression in this panel (see Fig. 1A), are even higher than in the parental cell line IGR39D. Only the transfectant with low exogenous c-myc expression, clone 6, shows a somewhat lower binding activity (Fig. 4A). Furthermore, binding to the MHC probe was also tested for protein extracts of four other melanoma cell lines, one with high HLA-B expression (518A2: low c-myc) and three with low HLA-B expression (603, 136−2 and 453A: high c-myc expression) (4). As in the case of the c-myc transfectants, these cell lines did not reveal a correlation between HLA-B expression and enhancer A binding activity (Fig. 4B).

To exclude the possibility that any effect of the c-myc oncogene on the binding activity of transcription factors KBF1 and NF-$\varkappa$B might be obscured by other MHC enhancer binding proteins in the gel retardation experiments, we had to determine the precise origin of the two specific retarded bands. Comparison with known KBF1-containing extracts (data not shown) had suggested that the lower band could be assigned to the enhancer A binding protein KBF1, which is a p50 homodimer (18,33). A possible candidate for the upper band was NF-$\varkappa$B, a heterodimer of p50 and p65 (18). The extracts were preincubated with polyclonal antisera against the subunit p65 (Santa Cruz Biotechnology, Santa Cruz, CA) and the subunit p50 (18). The results of these experiments with extracts of IGR39D and its c-myc-transfected derivatives confirmed our idea concerning the identities of the two retarded complexes. A representative experiment with a protein extract of c-myc transfectant clone 3 is shown in Figure 4C. Preincubation with the anti-p50 antiserum leads to supershifting of both complex A and complex B (Fig.4C, lane 3), while preincubation with the anti-p65 antiserum results in supershifting of only the upper band (Fig.4C, lane 4). This result is fully in concordance with binding of KBF1 (complex A) and NF-$\varkappa$B (complex B) to the MHC enhancer. The upper band present in all lanes (Fig.4C, marked with a dot) represents a complex of the large zinc finger protein MBP-2 and the MHC probe (34). This indicates that in the gel retardation assays no other proteins than KBF1, NF-$\varkappa$B and MBP-2 bind to the MHC enhancer.

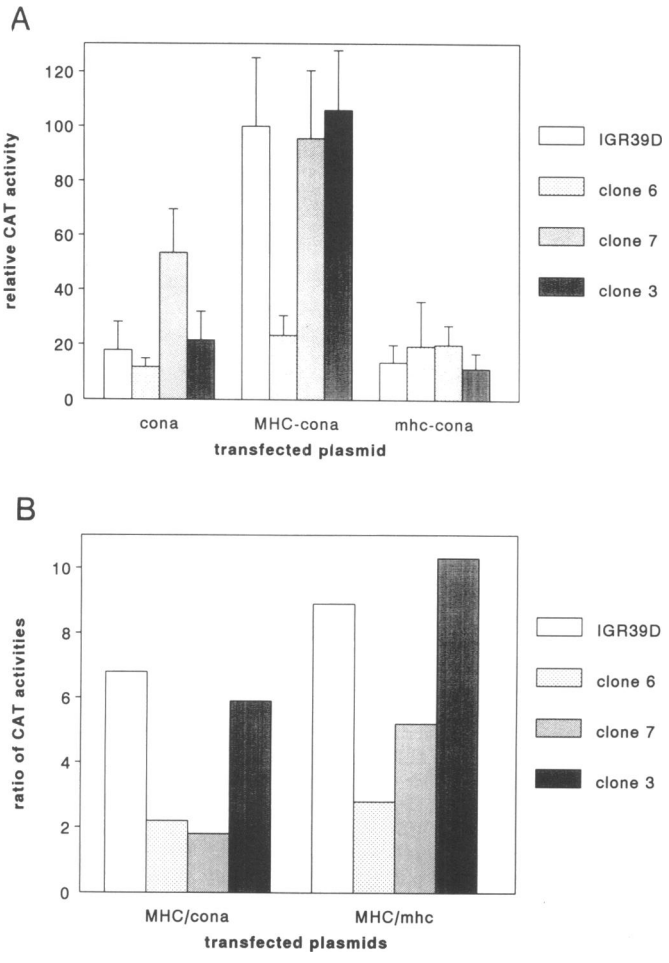

Figure 5. CAT activity in IGR39D and its c-myc transfectants after transient transfection of reporter constructs. **(A)** The plasmids conaCAT, MHC-conaCAT and mhc-conaCAT used to assay CAT activity in the various cell lines are indicated on the X axis. The cell lines IGR39D, its c-myc transfectants clone 6, 7 and 3 are indicated in the margin. CAT activities are given as the mean value obtained from four independent experiments and are expressed as the percentage of the value of the most active sample per assay. **(B)** The mean of the ratios of CAT activities per experiment was determined for the constructs MHC-conaCAT and conaCAT (MHC/cona) and for MHC-conaCAT and mhc-conaCAT (MHC/mhc) after transfection into the cell lines IGR39D and its c-myc transfectants clone 6, 7 and 3.

No correlation between functional activity of enhancer A and c-myc expression in transient transfection assays

Since gel retardation experiments only reflect the binding activity of cellular proteins *in vitro* and therefore may differ from activity of the particular enhancer *in vivo*, we measured the effect of c-myc expression on transcriptional stimulation of the MHC enhancer in transient transfection assays. To that end, we used chloramphenicol acetyl transferase (CAT) reporter constructs containing the chicken conalbumin promoter downstream of the enhancer A-region I element (MHC-conaCAT), and as controls the mutated element (mhc-conaCAT) or without enhancer (conaCAT, all plasmids kindly provided by I.Meijer (24)). The results of four independent transient transfection experiments are given as mean CAT activity normalized for the transfection efficiency by quantifying the activity of a co-transfected β-galactosidase reporter plasmid. The middle panel of Figure 5A shows that, as compared to the original cell line IGR39D, IGR-myc clone 6 generates a low activity of MHC-conaCAT. This might be related to the low binding activity found in clone 6 in gel retardation assays with the MHC enhancer as a probe (Fig.4A). However, clones 7 and 3, which have much lower levels of HLA-B expression than clone 6, display a high CAT activity, indicating that activity of the MHC enhancer and HLA-B expression are not at all correlated. The basal transcription levels of the plasmid conaCAT and the mutated control plasmid mhc-conaCAT are, as expected, low in all tested cell lines. One exception is the activity of conaCAT in clone 7, which was rather high (left panel). The effect of enhancer A *in vivo* can be expressed as the ratio of mean CAT activity of MHC-conaCAT over conaCAT or as the ratio of MHC-conaCAT over mhc-conaCAT. As can be seen in Figure 5B, these ratios vary considerably in the tested cell lines. However, no correlation can be found between c-myc (or HLA-B) expression and the ratio MHC/cona or MHC/mhc. Importantly, the level of stimulation of transcription by the MHC enhancer in IGR39D and the c-myc transfectant with the highest myc expression, clone 3, is almost identical, definitively excluding an effect of c-myc on binding of transcription factors to enhancer A. Therefore, we conclude that *in vitro* as well as in *in vivo* transient transfection experiments no impairment of enhancer A function by the c-myc oncogene occurs.

DISCUSSION

Modified expression levels of MHC Class I genes have frequently been observed in various types of human and animal tumors (2,3). Decreases in the expression levels of HLA Class I genes have been found in a high percentage of human melanoma tumors and coincide with activation of the c-myc oncogene. A causal relationship between c-myc expression and HLA Class I downregulation has been demonstrated by transfection of the c-myc gene into human melanoma cell lines. In melanoma, a preferential abrogation of HLA-B locus expression occurs (4,5). Another member of the myc oncogene family, N-myc, is capable of downmodulating the MHC Class I expression in neuroblastoma cells (6). The mechanism of this modulation has been unraveled: the transcription-activating function of the main enhancer of MHC Class I, enhancer A, is affected by overexpression of N-myc (22) and it has been shown that the level of expression of p50, of which two molecules form the KBF1 complex binding to the MHC enhancer, is transcriptionally downregulated by the N-myc oncogene (35). In addition, recent experiments established a role for proteins binding to enhancer A-region I in controlling the level of MHC Class I expression in Ad12 E1-transformed cells (24). In this article, we describe investigations on the functional activity of the HLA-B promoter region in human melanoma cells that express low or high amounts of the c-myc oncogene product. We show that the downmodulation by c-myc takes place at the level of transcription and involves the 666 bp promoter region of HLA-B.

As to get insight in the precise mechanism of downmodulation of HLA Class I genes by c-myc, we explored the involvement of the main MHC enhancer. In contrast to the status of this enhancer in neuroblastoma, unexpectedly, we could not establish any role for enhancer A in downmodulation of HLA Class I expression by c-myc. This was shown by two types of experiments. First, we tested the enhancer A binding activity present in melanoma cell lines and c-myc transfectants in gel retardation assays, and second, we investigated the functional

activity of this enhancer in transient transfection experiments. In both circumstances, no consistent correlation between function of the MHC enhancer and the level of c-myc expression could be found. It should be noted, however, that in two c-myc-transfected clones (clone 6 and clone 7) a decrease in stimulation of transcription by the MHC element can be detected, correlating at least in clone 6 with a lower binding activity in gel retardation experiments (see Fig. 4A and Fig. 5B). We have two obvious reasons to believe that these alterations in MHC enhancer activity are not at all responsible for the reduction of HLA-B expression. First, in IGR-myc clone 3, which expresses c-myc to a high level resulting in a very low level of HLA-B expression, the function of the MHC enhancer *in vitro* and *in vivo* appears to be unaffected as compared with the parental cell line. Thus, regulation of this enhancer is certainly not a general effect of c-myc. Second, the decrease in activity of the enhancer A element as detected in our experiments does not lead to an effect on HLA-A1 expression, because the level of expression of the HLA-A1 product in the three transfectants is almost the same as the expression found in the parental cell line (Fig. 1A). Since the sequence of the MHC enhancer is identical in nearly all thusfar sequenced HLA-A and HLA-B genes (including HLA-A1, Fig. 3), it is difficult to imagine how the HLA expression can be differentially regulated through this enhancer. Rather, the low activity found in the two above mentioned clones in transient CAT assays probably reflects a coincidental effect on the enhancer A element, lacking a physiological function in regulation of HLA Class I expression by c-myc. Based on these arguments, we conclude that enhancer A-region I is not responsible for the preferential downmodulation of HLA-B expression by c-myc in melanoma cells. This finding suggests that the mechanism of downregulation must involve some other region in the HLA Class I promoter. As a matter of fact, consistent nucleotide differences between HLA-A and HLA-B alleles in other parts of this region are found. Further *in vitro* and *in vivo* experiments with other regions of the HLA Class I promoter are in progress and should settle the mechanism of downregulation of HLA-B expression by the c-myc oncogene in human melanoma.

It is interesting to notice that c-, N- and L-Myc proteins are capable of forming heterodimers *in vitro* and *in vivo* with the basic-helix-loop-helix protein Max (36,37), which complex can specifically bind to the nucleotide sequence CACGTG (38). However, involvement of c-myc as a direct repressor of HLA Class I expression, *i.e* by binding to the promoter region of the HLA-B gene, can be ruled out since in the downregulated HLA promoter region no consensus Myc DNA binding site is present. Moreover, evidence has been provided that Myc/Max dimers function in mammalian cells as activators of transcription (39). This would indicate that c-myc overexpression induces an as yet unknown protein that is capable of repressing the transcription of HLA-B genes.

The finding that a role for enhancer A in the locus-specific downregulation by c-myc can be excluded implies that N-myc and c-myc act functionally different in neuroblastoma and melanoma, respectively, as far as the regulation of MHC Class I genes is concerned. This was already suggested by the finding that N-myc affects expression of all MHC Class I loci by inactivation of the MHC enhancer (6), whereas c-myc preferentially influences transcription of the HLA-B locus. Whether the two related oncogenes act via different pathways in the cell or whether the observed differences can be attributed to tissue-specific differences between neuroblastoma and melanoma remains to be studied.

ACKNOWLEDGMENTS

We thank Renske Steenbergen for initiating the CAT experiments, Ingeborg Meijer for providing the CAT constructs, Paul van der Logt for the antisera and René Bernards for the gift of the MHC probes and helpful discussions. We are grateful to A.G.Jochemsen, Laura van 't Veer and Andrea van Elsas for critical reading of the manuscript. This work was supported by grant IKW 89-11 from the Dutch Cancer Society.

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