

Suppression of MHC gene expression in cancer cells

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The class I antigens of the major histocompatibility complex play a crucial part in the recognition of foreign antigens by cytotoxic T lymphocytes. Some cancer cells exhibit a reduced expression of these antigens and this may help these cells to escape immune surveillance.

The class I antigens of the major histocompatibility complex (MHC) are highly polymorphic cell surface glycoproteins with molecular weights of approximately 45 000 Da. In the mouse, the classical class I antigens are encoded by three closely linked loci: H-2K, H-2D and H-2L. In man, class I antigens are also encoded by three loci: HLA-A, HLA-B and HLA-C. At the cell surface these molecules are non-covalently bound to a small non-polymorphic protein, β_2 -microglobulin¹. Expression of MHC class I antigens is first detected in the mid-somite stage of embryogenesis and continues in all nucleated cells of the adult organism, being highest in lymphoid organs and lowest in brain². The importance of these molecules resides in the fact that cytotoxic T lymphocytes (CTL) can only kill cells expressing a foreign antigen if these cells also express MHC class I antigens³. This implies that cancer cells that express tumor-associated antigens but have reduced expression of MHC class I antigens may be relatively resistant to CTL-mediated immune surveillance.

Suppression of MHC gene expression in rodent tumors

Evidence that attenuated expression of class I antigens can be advantageous for tumor cells comes mainly from rodent model systems. For example, a subline of the Gross leukemia virus-induced AKR leukemia cell line K36 was found to express class I genes encoded by the H-2D locus, but not those encoded by the H-2K locus. This cell line, named K36.16, is highly tumorigenic in syngeneic AKR mice. The importance of reduced H-2K expression to this particular tumor cell line was demonstrated by the fact that K36.16 cells in which the expression of the H-2K antigen had been restored by DNA-mediated gene transfer had lost to a large extent the ability to form tumors in immunocompetent syngeneic mice⁴.

Additional evidence to suggest that CTL-mediated immune surveillance can be involved in rejection of certain tumors comes from the study of the mouse T10 sarcoma. Here it was found that a highly metastatic clone of the T10 sarcoma, named IE7, lacked expression of the H-2K-encoded antigens. Reintroduction of the H-2K encoded antigens in the IE7 cells by DNA transfection was found to abrogate completely the metastatic ability of these sarcoma cells in immunocompetent mice. No reduction in metastatic ability was seen when the transfectants were injected into immunosuppressed mice, suggesting that H-2K-dependent immune mechanisms were responsible for the rejection of the metastases generated by the transfected T10 sarcoma cells⁵.

Genes responsible for suppression of MHC class I gene expression

The study of human adenoviruses provided the first evidence for the existence of specific genes that are capable of down-modulating the cell surface expression of class I histocompatibility antigens. In these studies, it was found that baby rat kidney cells

transformed by the highly oncogenic adenovirus 12 had greatly reduced expression of MHC class I antigens compared to cells transformed by the related non-oncogenic adenovirus 5. The ability of adenovirus 12 to suppress expression of class I antigens was shown to be a property of the viral E1a oncogene and was found to be intimately linked to the ability of the virus-transformed cells to form tumors in immunocompetent animals^{6,7}. The relation between the ability to suppress the expression of MHC class I genes and tumorigenicity has since then been further substantiated by Tanaka *et al.*⁸. They showed that reintroduction of the appropriate MHC class I gene under the control of a foreign promoter by DNA transfection abolished tumorigenicity of adenovirus 12-transformed cells.

As will be discussed in more detail below, the E1a oncogene shares the ability to suppress the expression of MHC class I genes with at least some members of the *myc* family of genes. This may suggest that the ability to modulate the expression of MHC class I gene expression is a general property of nuclear oncogenes.

The mechanism by which the E1a oncogene of adenovirus 12 suppresses the expression of MHC class I antigens has been studied by several groups. Schrier *et al.* have shown that the reduced cell surface expression of class I antigens on adenovirus 12-transformed cells is caused by a decrease in the cytoplasmic mRNA level of the class I heavy chain⁶. However, no decrease was found in the transcription rate of the class I genes in these cells, suggesting that E1a suppresses the expression of class I antigens at a post-transcriptional level⁹.

The effect of E1a on the expression of the MHC class I antigens has also been studied in transient expression assays using constructs in which a mouse class I gene promoter was fused to the bacterial chloramphenicol acetyltransferase (CAT) gene. The results obtained in these assays are inconclusive: some showed a suppressive effect of the E1a gene on the class I promoter in this assay¹⁰, whereas others did not show any effect of E1a on the hybrid gene¹¹. The precise mechanism by which the E1a gene down-modulates the expression of MHC class I antigens is therefore still not clear, although most of the data suggest a regulation at a post-transcriptional level.

Some adenoviruses can also employ a second, completely unrelated mechanism to reduce cell surface expression of MHC class I antigens. The 19 kDa early region 3 glycoprotein of adenovirus was found to associate within the cell with newly synthesized 45 kDa MHC class I polypeptides. This interaction

prevents terminal glycosylation of the class I molecules and consequently inhibits transport to the cell surface¹². This second mechanism is restricted to the non-oncogenic strains of adenovirus, suggesting that it does not contribute to the escape of virus-transformed cells from immune surveillance. It is possible, however, that the 19 kDa adenovirus-encoded protein plays a role in suppressing the immune response against virus-infected cells and thus may facilitate virus spread or help in the establishment of persistent viral infection.

Suppression of HLA expression in human cancers

Virus-associated cancers

Viruses play a role in the genesis of many of the rodent tumors that are used in the study of tumor immunology. Such virally-induced tumors often express one or more virus-encoded antigens at the cell surface, rendering these cells susceptible to destruction by the immune system of the tumor-bearing host. This antigenicity requires that the tumor cells employ some mechanism to evade the immune system of the host, suppression of MHC class I antigen expression being only one of them.

Of the many different forms of human cancers, only very few have been found consistently to harbor viruses. The best studied type of cancer in this class is Burkitt's lymphoma. In this particular type of cancer, Epstein Barr virus (EBV) is often expressed. One might therefore expect that such tumor cells would be sensitive to lysis by virus-specific cytotoxic T lymphocytes. It has been reported, however, that such virus-specific CTLs are not capable of killing virus-positive Burkitt's lymphoma cells, in spite of the fact that the same CTLs were capable of killing EBV-infected peripheral blood lymphocytes from the same patients¹³. This suggested that the Burkitt's lymphoma tumors possess some sort of mechanism to evade killing by CTL. Until recently it was believed that Burkitt's lymphoma tumor cells did not show any reduction in the expression of MHC class I antigens. Recent data, however, show that the resistance of Burkitt's lymphoma cells to CTL is likely to be caused by the specific down-regulation of an HLA-A-encoded gene expressed on the surface of these tumor cells¹⁴.

A different strategy appears to be used by another type of virus-associated human cancer, adult T-cell leukemia (ATL). In these tumors, HTLV I virus is often found to be expressed, but a reduction in the expression of MHC class I antigens has not been reported. It has been found, however, that the culture supernatants from ATL cell lines completely suppress B- and T-cell mitogenic responses. This corresponds with a complete failure of the immune system of ATL patients to respond to a variety of foreign antigens. Such inhibition of immune responses has been shown to be due to the secretion of a factor with an apparent molecular weight of 50–70 kDa by the ATL tumor cells¹⁵.

These examples once more emphasize the need of viral antigen-bearing tumor cells to evade the immune system of the host. The above-mentioned examples are exceptions, however, in that the vast majority of human cancers do not appear to have a viral etiology. Human cancers of non-viral origin are therefore con-

sidered to be much less antigenic than virally induced cancers and are not thought to have an equally great need to evade immune surveillance.

Spontaneous cancers

For many types of spontaneous human cancer, a few isolated cases have been reported in which a reduction in the expression of class I MHC antigens was observed. However, reduced expression of MHC class I antigen expression in human cancers is a rare phenomenon. The only tumor types in which low levels of MHC class I antigens have been found reproducibly are choriocarcinoma¹⁶, small cell lung cancer¹⁷ and neuroblastoma¹⁸. The choriocarcinoma tumors derive from trophoblasts, a highly specialized cell type of the placenta that is known not to express MHC class I antigens. Small cell lung cancer and neuroblastoma, on the other hand, do not derive from cells that are known to lack expression of MHC class I antigens, suggesting that specific regulatory mechanisms, developed during the course of tumorigenesis, operate in these two tumor types to suppress the expression of class I antigens. These two types of tumor are also different from most other forms of cancer in that they are often observed to carry amplified gene copies of one of the members of the *myc* gene family. The relationship between these two phenomena was recently demonstrated by showing that over-expression of the *N-myc* gene in a neuroblastoma tumor cell line by gene transfer caused a dramatic reduction in the expression of MHC class I antigens¹⁹.

Interestingly, clinical studies have shown that amplification of the *N-myc* gene is very rare in primary, non-metastatic neuroblastoma, and relatively frequent in metastatic neuroblastoma²⁰. This indicates that suppression of MHC class I antigen expression is not critical for the development of the primary tumor, but rather that it might be required for the successful metastatic spread of the neuroblastoma tumor cells. Additional evidence to suggest that down-modulation of MHC class I antigen expression is correlated with metastatic ability comes from studies on melanoma²¹ and breast cancer²². In these studies, it was found that metastases frequently expressed lower levels of class I antigens than the primary tumor from which they were derived. In the case of the melanomas, it appears that over-expression of the *c-myc* gene is responsible for the reduction of the expression of MHC class I antigens (P. I. Schrier, pers. commun.).

Suppression of MHC class I antigen expression thus seems to show a good correlation with metastatic ability of several human cancers. Whether suppressed expression of MHC class I antigens is sufficient to allow metastatic spread, however, can not be concluded as yet, since other mechanisms may contribute to this process. For example, over-expression of the *N-myc* gene in neuroblastoma cells was shown not only to cause a reduction in the expression of MHC class I antigens but also to result in a dramatic increase in the *in vivo* growth rate of the neuroblastoma cells¹⁹.

Cancers showing elevated MHC gene expression

The data discussed so far indicate a good correlation between growth rate of primary tumors or their

metastases and a reduction in the expression of MHC class I antigens. In several experimental systems, however, the opposite result was found. Thus, mouse B16 melanoma cells that had been selected for loss of MHC class I antigen expression *in vitro* were found to have a greatly reduced ability to form lung metastases compared with the parental, MHC-positive melanoma cells²³. Likewise, it was found that RBL-5 lymphoma cells that had been selected for loss of MHC class I gene expression had a greatly reduced ability to grow after subcutaneous injection into immunocompetent mice²⁴. In both experimental systems, the loss of expression of MHC class I antigens was paralleled by an increase in the sensitivity to lysis by natural killer (NK) cells. Moreover, some evidence suggests that the increase in sensitivity to NK cell-mediated lysis was in fact responsible for the reduced malignant behavior of the H-2-deficient tumor sublines^{23,24}.

These data show that in some tumor cells, loss of expression of MHC class I antigens is accompanied by a reduction in tumorigenicity. Such data suggest at the same time that an increase in the expression of MHC class I antigens in these particular tumor types could lead to an increase in tumorigenic or metastatic ability. Indeed, several tumors appear to express enhanced levels of MHC class I antigens when compared to the tissues from which they originate. Enhanced expression of class I antigens has also been observed in cells transformed by retroviruses²⁵, polyoma virus²⁶ and SV40 (Ref. 27). In the latter two cases, however, the increase in expression of class I antigens was observed when mouse cells were transformed *in vitro*, and it is not evident that this constitutes a growth advantage for the virus-transformed cells *in vivo*. In fact, when SV40-transformed cells were selected, by repeated passage *in vivo*, for increased ability to form tumors in immunocompetent mice, it was found that the cells having increased *in vivo* growth rate had lost expression of H2-K-encoded antigens²⁸. This suggests that enhanced expression of MHC class I antigens seen *in vitro* may actually be responsible for the limited ability of SV40- and polyoma-transformed cells to grow in immunocompetent hosts.

Conclusions

Table 1 summarizes the relationship between the

level of expression of MHC class I antigens and tumor characteristics for a variety of cancers. Clearly, the interaction between the immune system and tumor cells is dynamic and complex, depending upon a number of different immunological effector mechanisms. The activity of at least two types of effector cell appears to be dependent on the expression of MHC class I antigens by the target cells. Cytotoxic T lymphocytes require expression of MHC class I to kill target cells, whereas other evidence suggests that high levels of MHC class I antigens inhibit natural killer cells in their ability to kill tumor cells. The opposing responses of these two effector cells make it hard to predict the effect of altered MHC expression on tumor cells. A consensus would be that tumors of viral etiology are good targets for CTL and it is likely that these tumors will benefit from a reduction in the expression of MHC class I antigens.

In human cancers of non-viral origin, reduced expression of MHC class I antigens seems to be associated mostly with an increased ability of tumor cells to metastasize. This may be caused by the fact that spontaneous tumors express tumor-associated antigens which, in comparison to viral antigens, are poor targets for CTL. CTL may therefore be relatively ineffective against a large tumor mass at the primary site. However, by the time tumor cells begin to metastasize, the primary tumor has presented the tumor-associated antigens for a prolonged period to the immune system, thus stimulating a response. Metastatic tumor cells, which usually travel as single cells or in small clusters, may therefore be relatively susceptible to immune destruction in the blood stream. As a consequence, reduced expression of MHC class I antigens may help metastatic tumor cells to establish successfully at a secondary site.

It is conceivable that the use of agents, such as interferons, that stimulate the expression of MHC class I antigens may be effective in reducing the rate at which certain tumors metastasize. On the other hand, increased expression of MHC class I antigens might lead to resistance to natural killer cell-mediated immune surveillance and thus favor tumor outgrowth. In one case, it has actually been shown that treatment of tumor cells with γ -interferon caused an increase in metastatic ability — this in spite of the growth-

Table 1. Association between MHC class I antigen expression and tumor characteristics in some rodent and human cancers

Animal	Tumor	Associated virus	Level of MHC class I antigens	Effects on tumor	Ref.
Mouse	K36 leukemia	Gross leukemia virus	H-2K absent	Increased tumorigenicity	4
Mouse	T10 sarcoma	—	H-2K absent	Increased metastasis	5
Rat, mouse	Sarcoma	Adenovirus 12	Decreased	Increased tumorigenicity	6,7
Human	Burkitt's lymphoma	EBV	HLA-A decreased	Resistance to virus-specific CTL	14
Human	Neuroblastoma	—	Decreased in N-myc amplified tumors	Increased metastatic ability	18,19
Human	Breast cancer	—	Decreased in metastases	Increased metastatic ability?	22
Human	Melanoma	—	Decreased in metastases	Increased metastatic ability?	21
Human	Small cell lung cancer	—	Decreased	Increased metastatic ability?	17
Mouse	B16 melanoma	—	Decreased	Decreased metastatic ability, increased susceptibility to NK cells	23
Mouse	RBL-5 lymphoma	—	Decreased	Decreased metastatic ability, increased susceptibility to NK cells	24

▲ MHC class I
■ Tumor antigen

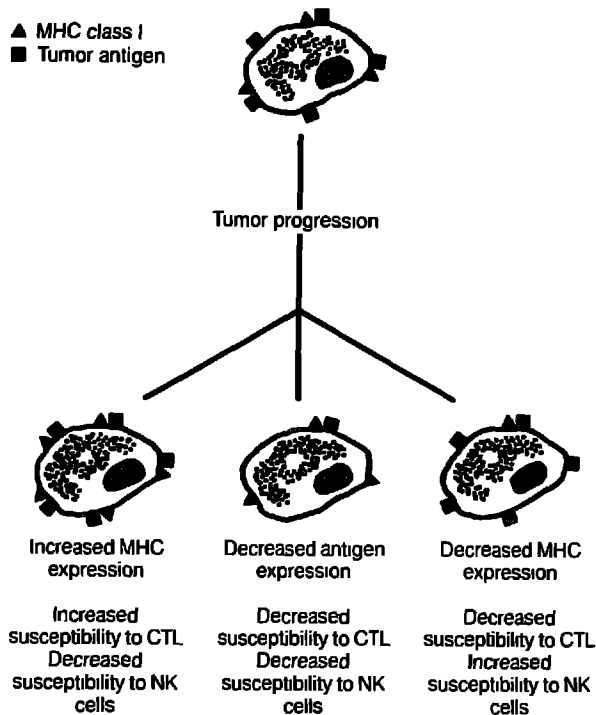


Fig. 1. Antigenic changes during tumor progression. Cytotoxic T lymphocytes (CTL) can only kill cells expressing a foreign antigen if those cells also express MHC class I molecules. A decrease in the expression of MHC class I antigens on tumor cells therefore leads to a decrease in the susceptibility of the tumor cells to antigen-specific CTL. An increase in the expression of MHC class I antigens on the tumor cells leads to increased recognition by antigen-specific CTL. Natural killer (NK) cells respond differently to altered expression of MHC class I molecules on tumor cells. In several systems NK cells have been found to be inhibited in their ability to lyse tumor cells by high levels of MHC class I antigens on the target cells and stimulated in lytic activity by low levels of MHC class I antigens. A third possibility, not discussed in this paper, is that the tumor cells might lose the expression of one or more of the antigens that are recognized by immune effector mechanisms.

inhibitory effect that the drug exerted on the tumor cells²⁹.

The effect of suppression of MHC class I antigen expression is thus likely to be dependent on the nature of the tumor-associated antigens expressed by the tumor cells. If the antigens expressed at the cell surface are such that the tumor cell is a potential target for CTL, a reduction in the expression of class I antigens is likely to be beneficial for the tumor cell. If the cell is a potential target for NK cells, an increase in the expression of MHC class I antigens is likely to favor tumor outgrowth. Some of the possible effects of antigenic changes during tumor progression are shown in Fig. 1.

The factors that regulate susceptibility to the different types of immune effector cell are largely unknown. Recent evidence, however, begins to suggest that cellular oncogenes can play an important part in this process. Thus, enhanced expression of the *myc* family of oncogenes seems to reduce susceptibility of certain human cancer cells to CTL¹⁹. In contrast, it appears that over-expression of MHC class I antigens can in a number of cell types be induced by the *fos* oncogene

(M. Feldman, pers. commun.). Furthermore, it was shown that transfection of a *ras* oncogene into a mouse fibroblast cell line rendered these cells susceptible to NK cell-mediated lysis³⁰. These observations were made in a few selected cell types and more work is required to assess whether these findings have general validity.

Although the interactions between tumor cells and the immune system are only beginning to be understood, it is likely that the techniques of molecular biology will greatly help in leading to an understanding of this complex process.

Acknowledgements

I thank Robert Weinberg for critical reading of this manuscript. The author is supported by a grant from the Netherlands Organization for the Advancement of Pure Research (ZWO).

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