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CIITA Transformation Rescues the Apoptotic Function of MHC Class II in Melanoma Cells

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Abstract. *Background: Cell-surface major histocompatibility (MHC) class II molecules contribute to a molecular, inter-cellular complex that stimulates T-cells. MHC class II molecules also activate signaling pathways leading to apoptosis. Lack of CIITA, a co-activator of the MHC class II gene promoter, is responsible for lack of MHC class II on most of the MHC class II-negative melanoma cell lines. Materials and Methods: We rescued CIITA and MHC class II expression in melanoma cell lines by stable transformation with a CIITA expression vector and assayed for MHC class II-mediated apoptosis. Results: Reconstitution of CIITA in multiple CIITA-negative melanoma lines restores the apoptotic function of melanoma MHC class II. Conclusion: Lack of MHC class II transcription, rather than defects in MHC class II protein function or defects in the other components of the MHC class II-stimulated apoptotic pathway, prevents MHC class II-mediated apoptosis in melanoma cells.*

Major histocompatibility (MHC) class II molecules have been extensively studied as antigen-presenting molecules and for their role in stimulating an immune response. In particular, MHC class II molecules are ordinarily expressed on a subset of specialized immune function cells, termed professional antigen-presenting cells (APC), such as dendritic cells, that gather antigen from the extra-cellular environment for processing, for loading onto MHC class II, and for surface expression of the MHC class II-antigen complex. Interaction of the complex with the T-cell receptor leads either to activation of CD4-positive T-cells or to T-cell anergy, in the absence of a co-stimulatory molecule on the MHC class II-bearing cell.

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MHC class II molecules are also expressed on numerous other mesenchymal and parenchymal cell types in response to IFN-γ and, in some cases, these cells are able to present antigen and stimulate T-cells. However, the role of MHC class II on a "non-professional" APC remains controversial. The tumor cells that arise from mesenchymal and parenchymal cells can either remain inducible for MHC class II or be non-inducible (1). The non-inducible phenotype has been shown to be due to tumor-related mutations (2-4).

Melanoma cells represent a unique category of MHC class II-expressing cells in that a subset of melanoma cell lines constitutively express MHC class II. This is a curious phenotype because melanoma cells do not obviously arise from professional APC's, the only normal cell types that constitutively express MHC class II. We have determined that about fifteen percent of melanoma cell lines are MHC class II-positive (5), and others have reported that as many as sixty percent of melanoma cells lines are MHC class II-positive (6). We and others have determined that MHC class II-positive melanoma cells also express CIITA, which activates the MHC class II promoter in almost all other cells types (5, 7). Also, lack of CIITA is the reason for the lack of MHC class II-negative melanoma cell lines (5). Here, we report that MHC class II expression in these cell lines is rescued by CIITA transformation, confirming that the lack of MHC class II expression is traceable to the lack of CIITA expression. Furthermore, CIITA-mediated rescue of MHC class II expression also rescues the apoptotic function of MHC class II (8), indicating that no other part of the apoptotic signaling pathway has been affected in the indicated melanoma cells.

Materials and Methods

Cell lines, tissue culture and generation of CIITA transformants. The MHC class II constitutive and MHC class II-negative melanoma cell lines and propagation of the cell lines have been previously, extensively described (5). The CIITA transformants were generated using a previously described CIITA expression vector (9) and selection for G418-resistant transformants. Control transformants were generated using the empty vector, pRC-RSV (Invitrogen).

Table I. *Cell lines and melanoma transformants used in this study.*

Cell line	Description	HLA-DR status
Raji (HLA-DR constitutive B-cell line)	EBV transformed B-cell line	HLA-DR constitutive
WM9	Patient-derived melanoma cell line	HLA-DR constitutive
1102-mel	Patient-derived melanoma cell line	HLA-DR constitutive
1286-mel	Patient-derived melanoma cell line	HLA-DR-negative due to lack of constitutive CIITA expression
1286-mel-empty vector-1	Control transformant of 1286	Negative
1286-mel-empty vector-2	Control transformant of 1286	Negative
1286-mel-CIITA-1	1286 transformed with a CIITA expression vector	Positive
1286-mel-CIITA-2	1286 transformed with a CIITA expression vector	Positive
1286-mel-CIITA-3	1286 transformed with a CIITA expression vector	Positive

MHC class II cross-linking and apoptosis assays. L243 anti-HLA-DR hybridoma cells, and hybridoma cells secreting an isotype control antibody, were cultured for 24 hours with RPMI media supplemented with 10% fetal bovine serum, 5 ml of 200 mM L-glutamine, 5 ml of 5000 units/ml penicillin, 5 ml of streptomycin (5,000 μg/ml) and 5 ml of 100 mM sodium pyruvate (Gibco). The hybridoma culture media was recovered and applied to plated melanoma cells. The melanoma cells were incubated with the hybridoma media containing secreted antibody for a 24-hour period before flow cytometry analysis.

Flow cytometry assays for surface HLA-DR and propidium iodide uptake. For surface HLA-DR detection, cells were washed with PBS and incubated with 2 ml of Enz-Free solution for 15 minutes. Cells were collected by centrifugation and resuspended in 500 μl of PFA solution (1% fetal bovine serum, 0.02% NaN_3 in PBS). Samples were incubated on ice for 30 minutes, recovered by centrifugation and resuspended in 500 μl of PFA. Anti-DR or isotype control was added to the cell suspension. After 45 minutes, cells were recovered by centrifugation and resuspended in PBS for analysis by flow cytometry. Antibodies for surface DR detection: FITC anti-human HLA-DR (Pharmingen, Cat. # 32414X) and FITC mouse IgG_{2a}, Î isotype control (Pharmingen, Cat. # 33804X) were used at concentrations of 10 μg/ml.

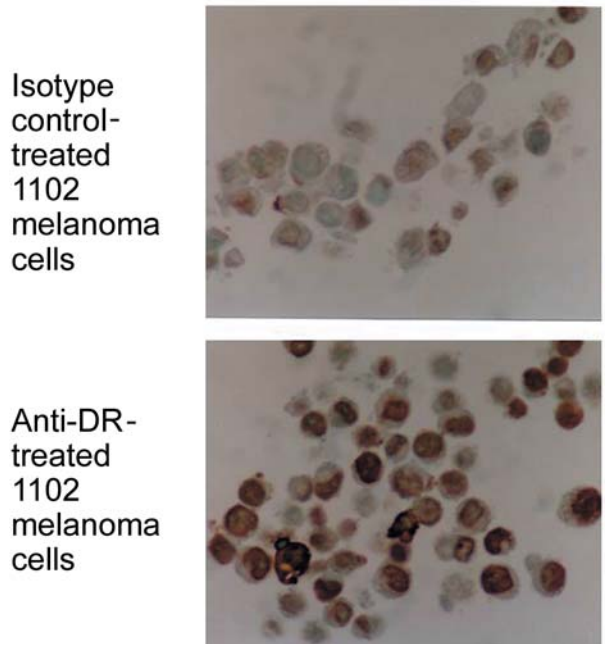

Figure 1. *TUNEL assay of isotype–or anti-HLA-DR–treated 1102 melanoma cells. A sister culture of cells was also assayed for propidium iodide uptake, as a quantitative measure of cell death (Table II)*

For determination of propidium iodide uptake, cells were incubated with 2 ml of Enz-Free (Gibco) for 15 minutes and collected by centrifugation. Cells were resuspended in 1 ml of PBS. Samples were stained for 10 minutes with 100 μg/ml of propidium iodine and analyzed by flow cytometry.

Results and Discussion

MHC class II cross-linking leads to apoptosis in a variety of normal, immune function cell types. To verify this result for melanoma cells, we treated the MHC class II constitutive 1102-mel cells (Table I) with the anti-HLA-DR monoclonal antibody L243 or with an isotype control. HLA-DR is the best studied, most immunologically important and most highly expressed human MHC class II molecule. The L243 treatment led to apoptosis of the 1102-mel cells as determined by the TUNEL assay (Figure 1) and by propidium iodide (PI) uptake (Table II), used extensively as confirmation of apoptosis by MHC class II cross-linking (8, 10-12). In particular, we normalized the PI uptake to the level of HLA-DR surface staining, which indicated that the apoptotic effect of L243 on the melanoma cells was equal to the L243 apoptotic effect on the Raji B-cells, previously shown to undergo apoptosis in response to L243 (Table II).

Table II. *Amount of propidium iodide uptake, following HLA-DR cross-linking, as a function of surface HLA-DR*[a].

Cell line	Ratio of PI uptake to HLA-DR surface expression
Raji	6.1
WM9	5.2
1102	5.0

[a] HLA-DR expression and PI uptake levels were measured by flow cytometry as in Figure 2; Fifty-six percent of Raji cells took up propidium iodide, *i.e.*, were undergoing cell death.

We have described a series of MHC class II-negative melanoma cells that lack the co-activator for the MHC class II promoter, CIITA (5). We generated a series of CIITA transformants of one of these MHC class II-negative melanoma lines, 1286-mel, as well as control transformants, designated 1286-mel-empty vector-1 and -2, respectively (Table I). Transformation with the CIITA expression vector rescued HLA-DR surface expression (Figure 2; Tables I, III), indicating that lack of CIITA is indeed responsible for lack of surface MHC class in the MHC class II-negative 1286-mel.

The 1286-mel CIITA transformants and the control transformants were treated with L243 or an isotype control

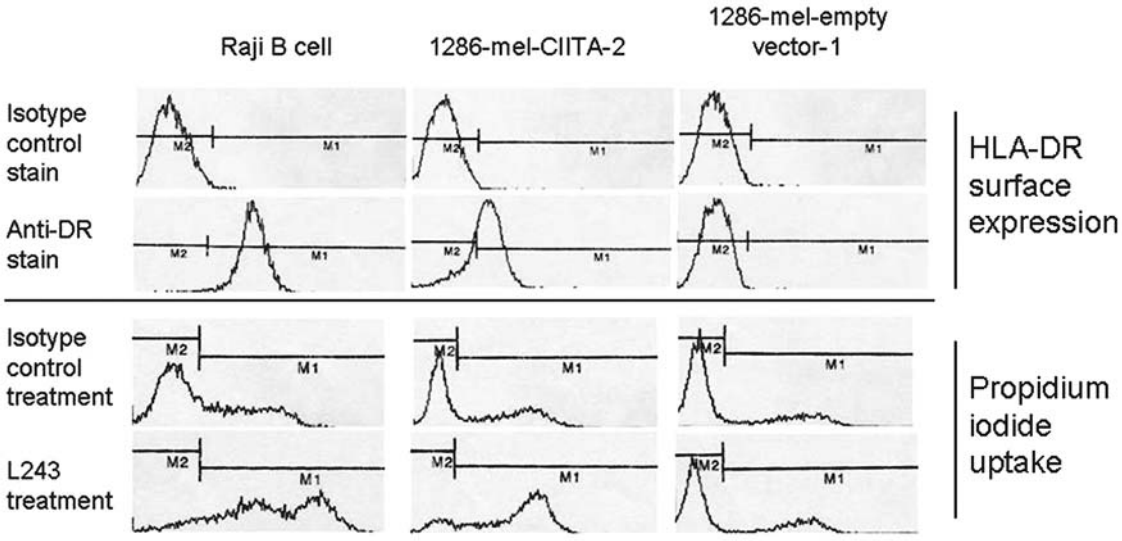

Figure 2. *Example flow cytometry data summarized in Table III. Indicated cell lines are described in Tables I and III.*

Table III. *CIITA-transformed 1286 melanoma cells susceptible to HLA-DR cross-linking-mediated apoptosis.*

Cell line	% staining for isotype control	% staining for HLA-DR	% take-up of PI when treated with an isotype control antibody	% take-up of PI when treated with an HLA-DR cross-linking antibody (L243)	Ratio of % take-up of PI with anti-DR treatment *vs.* isotype control treatment
Raji (HLA-DR constitutive B-cell line)	1.04	98.52	39.31	90.32	2.30
1286-mel-empty vector-1	0.31	0.12	21.13	23.83	1.13
1286-mel-empty vector-2	0.58	0.09	51.29	45.90	0.89
1286-mel-CIITA-1	ND	ND	**19.88**	**88.56**	**4.45**
1286-mel-CIITA-2	0.73	74.47	**42.30**	**85.94**	**2.03**
1286-mel-CIITA-3	0.47	27.31	**29.25**	**51.85**	**1.77**

antibody. The CIITA transformants, but not the control transformants, underwent cell death in response to the anti-DR L243 (Figure 2, Table III).

The above data indicate that lack of MHC class II surface expression alone prevents MHC class II-mediated apoptosis. These data indicate that no other part of the MHC class II-mediated apoptosis pathways is defective or lost in the 1286-mel cells. In one model of melanoma development (5), the MHC class II-negative cells arise from MHC class II-positive cells through the loss of CIITA promoter function. Such a process may facilitate tumor growth unimpeded by MHC class II-mediated apoptosis, which in turn could be activated by binding of the MHC class II to the T-cell receptor or to an unknown ligand. Furthermore, MHC class II-mediated apoptosis has been proposed as antitumor therapy, and the above data suggest that rescue of MHC class II expression may facilitate the use of such a therapy for MHC class II-negative, CIITA-negative melanomas.

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