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ScienceDirect
Experimental and Molecular Pathology 82 (2007) 68–76
Experimental and Molecular Pathology
www.elsevier.com/locate/yexmp

Inhibition of MHC class II gene expression in uveal melanoma cells is due to methylation of the CIITA gene or an upstream activator

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Received 7 March 2006
Available online 2 May 2006

Abstract

Most cells with an intact interferon-gamma receptor and signaling pathway are able to express MHC class II molecules when treated with cytokines such as interferon-gamma and tumor necrosis factor-a. Interestingly, primary uveal melanocytes and most ocular melanoma cells are resistant to interferon-gamma mediated induction of class II MHC genes. This unusual phenotype is hypothesized to be germane to the immune-privileged status to the eye. Via a series of experiments, we have probed the molecular basis of this class II MHC resistant phenotype. We have analyzed the methylation status of the gene encoding the class II transactivator (CIITA), and asked whether treatment of class II MHC resistant ocular melanoma cells with the demethylating agent 5′-azacytidine can restore interferon-gamma inducibility of these class II MHC genes in these cells. The data obtained suggest that the specific blockade in cytokine-induced class II MHC gene expression is due to a suppression of the gene encoding the class II transactivator (CIITA). Treatment with 5′ azacytidine restores the ability of these cells to express class II MHC genes upon interferon-gamma treatment. Whilst this is reminiscent of what occurs in another immune-privileged tissue – the placental trophoblast – we show here that silencing of the CIITA gene in uveal melanocytes either involves methylation of distinct nucleotides from those detected in trophoblasts, or involves an upstream activator of CIITA gene expression.

Introduction

The eye is an immunologically privileged site where inflammation mediated by CD4+ T cells is strongly inhibited. This "deviant" immunity is thought to confer a competitive advantage, as inflammatory responses in the eye obscure vision. This regional immunity also makes the eye a remarkably successful site for allografts, with corneal transplants enjoying very high rates of long term success. This unique environment of the eye was first demonstrated by Medawar approximately 50 years ago, and is now known to also occur in other tissues such as at the maternal/fetal interface, the testes and the brain. Work from a number of laboratories has now revealed a number of molecular mechanisms that contribute to the generation of immune privilege. These include (i) a blood–tissue barrier that restricts access of sensitized lymphocytes, (ii) atypical lymphatic drainage that allows antigens and APC to escape directly into the blood vasculature, (iii) a unique local environment that contains immunosuppressive factors that regulate both the induction and the expression of cell-mediated immunity, (iv) the constitutive expression of Fas Ligand on certain ocular tissues that induces apoptosis if infiltrating Fas-positive lymphocytes, and (v) a reduced number of "mature" class II MHC-positive antigen presenting cells located in the uninflammed tissue, and (vi) a failure of cells within these tissues to express class II MHC molecules when exposed to cytokines such as interferon-g.

We and others have previously found that both primary uveal melanocytes and most ocular melanoma cells (derived from uveal melanocytes) fail to express class II MHC molecules on their surface when exposed to interferon-g (Radosevich et al., 2004). This phenotype results from either epigenetic silencing of the endogenous CIITA gene, or to post-transcriptional inhibition of class II MHC polypeptide synthesis. Corneal endothelial cells also require multiple cytokines to induce class II MHC gene expression, indicating that multiple cell types in the eye may share this interesting MHC phenotype (Arancibia-Carcamo et al., 2004).

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0014-4800/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexmp.2006.03.005

As we have previously found that both primary uveal melanocytes and many ocular melanoma cell lines are resistant to interferon-g mediated induction of class II MHC genes due to epigenetic silencing of the CIITA genes, we have probed the molecular basis of this CIITA gene silencing in several ocular melanoma cell lines. These have been used since they can be propagated more easily than primary uveal melanocytes, and are amenable to reporter gene transfection and biochemical studies. Since ocular melanoma cells are derived from the malignant transformation of normal uveal melanocytes, it is perhaps not surprising that most ocular melanoma cell lines and primary uveal melanocytes have in common the resistance to IFN-g mediated induction of class II MHC genes.

The relevance of this MHC phenotype to immune privilege is supported by the fact that both primary skin melanocytes and most skin melanomas are able to express class II MHC molecules in response to IFN-g. Indeed, approximately 60% of primary skin melanomas constitutively express class II DR. This striking difference in MHC phenotype between an immune privileged (eye) and immune competent (skin) melanocyte strongly suggests that the MHC-resistant phenotype is germane to immunologic privilege. Finally, the finding that other immune-privileged tissues (e.g. trophoblasts and brain tissue) also exhibit unusual MHC phenotypes (class I MHC or class II MHC-negative) adds further evidence that modulation of MHC gene expression can contribute to the immunogenicity of the tissue. Indeed, data from several laboratories indicate that class II-positive primary skin melanoma cells function as antigen presenting cells and under stimulate proliferation and lymphokine secretion of autologous CD4+ T cells. This does not occur with IFN-g treated uveal melanocytes.

We have recently found that most ocular melanoma cells do not constitutively express CIITA transcripts and are resistant to IFN-g induced expression of CIITA (Radosevich et al., 2004). This is markedly different from the phenotype of skin melanomas, and also correlates well with their inability to express class II MHC molecules on the surface. This blockade in CIITA transcription is the only barrier to class II antigen expression in the majority of ocular melanoma cell lines, as ectopic expression of CIITA results in the display of class II MHC molecules on the cell surface. The defect in CIITA gene expression in these cells is not due to a defective IFN-g signaling pathway, as IFN-g treatment of the cells induced the expression of class I MHC molecules, and heterologous CIITA reporter constructs. We therefore concluded that the blockade in CIITA transcription and class II MHC expression in ocular melanomas, and likely uveal melanocytes, is due to an epigenetic silencing of the endogenous CIITA gene. The data presented in this paper support that conclusion.

There has long been evidence that methylation of DNA can effect gene regulation. In some cases there is an increase of gene expression (for example in some cases of class I MHC gene expression) but in most instances methylation results in an inhibition of gene expression. This negative affect on transcription is due to the methylation of CpG dinucleotides. The methylation has been shown to prevent transcription factor access to the DNA (both in vitro and in vivo) and can also recruit repressors that bind methylated CpGs (Kass et al., 1997). The critical role for methylation in the silencing of genes is demonstrable by the restoration of gene activity upon incubation with 5-aza-2′-deoxycytidine (5AC) which is an inhibitor of DNA methyltransferase (Razin and Cedar, 1991). We show in this paper that the epigenetic silencing of CIITA gene induction by IFN-g is accomplished by the either the methylation of the CIITA gene or an essential upstream activator of CIITA. This is reminiscent of the findings by Boss, Tomasi and van den Elsen focusing on fetal trophoblasts, although the precise mechanism of methylation-mediated silencing is shown to be distinct (Murphy and Tomasi, 1998; Morris et al., 1998, 2000; van den Elsen et al., 2000, 2001).

Materials and methods

Tissue culture

HeLa cells were grown in DMEM supplemented with 10% heat-inactivated FCS, and 100 U/ml penicillin and streptomycin. Melanoma 202 cells were obtained through Bruce Ksander at the Schepens Eye Research Institute, Boston, MA. Other ocular melanoma cell were obtained from Martine Jager. We thank both Drs. Ksander and Jager for making these cells available to us for these investigations. Ocular melanoma cell lines were established using techniques previously described. Tumor tissue was dissected from surrounding normal uveal tissue and enzymatically digested to yield a single cell suspension in a petri dish containing 10 ml of collagenase at 150 units/ml (Sigma, St. Louis, MO) in complete RPMI 1640 (BioWhittaker, Wakersville, MD) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT) 2.0 mM glutamine (BioWhittaker), 100 U/ml of penicillin and 100 U/ml of streptomycin, 0.1% fungizone (BioWhittaker), 0.01 M HEPES (N-2-hydroxy-ethylpiperazine-N′-2 ethanesulfonic acid, BioWhittaker), and 0.5% 2-β-mercaptoethanol (Sigma). Tissue was incubated for 90 min at 37°C after which the released cells were removed and the debris allowed to settle. Cells in the culture supernatant were recovered, washed 3 times, and examined microscopically for the presence of viable tumor cells. Ocular melanoma cells were maintained in complete RPMI 1640 culture medium as described. Cells were incubated at 37°C with 5% CO2 and passaged when they reached a confluent monolayer by treatment with trypsin-EDTA (BioWhittaker). Tumor cell lines were demonstrated to be ocular melanoma cells by the expression of MAGE (Melanoma Antigen Genes) that are transcriptionally active only in melanoma cells and are not active in normal non-malignant cells. Jar (Obtained from American Type Culture Collection, HTB-144) is a choriocarcinoma cell line that does not express MHC class II genes upon stimulation with IFN-gamma and was maintained in RPMI 1640 supplemented with 10% heat-inactivated FCS, and 100 U/ml penicillin and streptomycin.

PCR amplification of CIITA promoters

Genomic DNA of melanoma 202 cells and Jurkat cells (a cell line that is known to express class II genes after incubation with interferon-gamma) was isolated by using a Qiagen genomic DNA isolation kit. The entire region of promoter IV was amplified by PCR and was then sequenced at the Tufts DNA sequencing lab. The interferon-gamma responsive region of promoter III was also amplified by PCR. The PCR products were then run on a 1% agarose gel and analyzed on a gel documentation system.

Southern blot

Genomic DNA was extracted from cultured cells using a Qiagen genomic DNA purification kit (Qiagen Inc., Valencia, CA) and following the manufacturer's protocol. Restriction endonuclease digested DNA was separated on 1.0% agarose gels and capillary transferred onto positively charged nylon membranes (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire,