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Constitutive and IFNγ-induced activation of *MHC2TA* promoter type III in human melanoma cell lines is governed by separate regulatory elements within the PIII upstream regulatory region

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Abstract

Cell lines established from tumor tissue of cutaneous melanoma biopsies often display constitutive and IFNγ-inducible expression of MHC class II molecules. The expression of MHC class II molecules in melanoma is associated with an overall poor prognosis and unfavorable clinical outcome. We have analyzed the DNA elements and interacting transcription factors that control the constitutive and IFNγ-inducible expression of the class II transactivator (CIITA), a co-activator essential for transcription of all MHC class II genes. Our studies reveal the activation of multiple CIITA promoter regions (CIITA-PII, -PIII and -PIV) in melanoma cell lines for both the constitutive and IFNγ-inducible expression of MHC class II molecules. Furthermore, we show that constitutive and IFNγ-inducible expression of the CIITA-PIII isoform is governed by separate regulatory elements within the PIII upstream regulatory region (PURR). Similarly constitutive activation in melanoma of CIITA-PII, CIITA-PIII, and CIITA-PIV does not require components of the IFNγ signaling pathway. However, these components are readily recruited to the PURR and CIITA-PIV after exposure of cells to IFNγ and account for the IFNγ-induced expression of CIITA. Together, our data reveal the contribution of distinct elements and factors in the constitutive and IFNγ-inducible expression of CIITA in melanoma cell lines of the skin.

Keywords: MHC; Gene regulation; Transcription factors; Chromatin; Melanoma

1. Introduction

Melanoma of the skin is a highly malignant cancer type, which is classified into discrete stages of biological progression representing early transformed cells to finally a highly invasive and often fatal tumor (Chin, 2003). The development and progression of human cutaneous melanoma reflects the accumulation of irreversible alterations in an unknown number of genes that confer changes in protein expression patterns and growth advantage. It has been demonstrated that expression of major histocompatibility complex (MHC) class II molecules on primary melanoma is associated with an overall poor prognosis and unfavorable clinical outcome (Wilson et al., 1979; Houghton et al., 1982; Elder et al., 1989; Brogelli et al., 1990; Barnhill, 1993; Ruiter and Brocker, 1993; Ostmeier et al., 2001). To date it is unclear whether the association of MHC class II gene expression on melanomas with tumor progression is due to a direct role of these molecules or caused by intracellular events that promote tumor survival and simultaneously lead to activation of MHC class II genes.

The constitutive expression of these antigen-presenting receptors in melanoma is highly unusual because under normal circumstances constitutive expression of MHC class II molecules is restricted to antigen presenting cells (APCs) of

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the immune system such as dendritic cells (DCs) and B lymphocytes. This is because activation of $CD4^+$ T cells is mediated through interaction of the T cell receptor (TR) with peptide/MHC class II complexes expressed on the cell surface of APCs. This is a prerequisite for induction of an antigen-specific immune response (Levin et al., 1993; Guery et al., 1996). In this way, $CD4^+$ T cells are also central to anti-tumor immunity because they help to initiate and maintain the MHC class I-mediated CTL response against the tumor. Therefore, it could be argued that due to their constitutive MHC class II expression melanoma cells represent non-professional APCs and as such could influence the anti-tumor immune response.

It is well established that the class II transactivator (CIITA), encoded by the *MHC2TA* gene, is the master regulator of MHC class II gene expression and participates in the regulation of the accessory genes (invariant chain (Ii), HLA-DM and HLA-DO) (Chang et al., 1994; Nagarajan et al., 2002; Steimle et al., 1993; Taxman et al., 2000). Besides the constitutive expression in APCs, CIITA and congruent MHC class II expression can also be induced in many other cell types by inflammatory cytokines of which interferon-γ (IFNγ) is the most potent (Collins et al., 1984; Ting and Baldwin, 1993; Rohn et al., 1996). The transcriptional regulation of *MHC2TA* is controlled by a 14 kb multi-promoter region that harbors four independent promoter units each transcribing a unique first exon (Muhlethaler-Mottet et al., 1997). Promoter PI (CIITA-PI) is solely utilized for the expression in DCs, whereas PIII (CIITA-PIII) is active in B lymphocytes, DCs, monocytes and human activated T cells (van den Elsen et al., 2004). CIITA-PIV has been shown to be the promoter predominantly involved in IFNγ-inducible *MHC2TA* expression whereas the function of CIITA-PII is still poorly understood. In addition to CIITA-PIV, IFNγ can also activate the CIITA-PIII promoter region in human non-hematopoietic cells, like fibroblasts and epithelial cells. This activation has been shown to be mediated through a 4 kb PIII upstream regulatory region (PURR) located 2 kb upstream of the CIITA-PIII core promoter region (Piskurich et al., 1999). It should be noted that whereas the PURR region is involved in the IFNγ induction of CIITA-PIII in non-hematopoietic cells, it does not influence the constitutive expression of CIITA-PIII in established B cell lines (Piskurich et al., 1999). Because of the apparent lack of constitutive MHC class II molecule expression in non-hematopoietic cells, it is striking that human melanoma tumor cells commonly constitutively express MHC class II molecules at their cell surface. This MHC class II expression in melanoma cells is governed by the constitutive transcription of the CIITA-PIII and CIITA-PIV elements (Goodwin et al., 2001; Deffrennes et al., 2001). In addition, it has been shown that constitutive and IFNγ-induced transcriptional activation of CIITA-PIII in melanoma cells is mediated through the 4 kb PURR (Deffrennes et al., 2001).

To further define the elements and factors that are involved in melanoma-mediated expression of the *MHC2TA* gene, we compared in this study the constitutive and IFNγ-induced activation of all *MHC2TA*-promoters (CIITA-PI, -PII, -PIII and -PIV) in a large panel of well-characterized (with respect to MHC class II molecule expression profile) patient-derived cutaneous melanoma cell lines (Versteeg et al., 1988; Ramirez-Montagut et al., 2000; Kurnick et al., 2001). Moreover, we evaluated whether constitutive activation of *MHC2TA* transcription in melanoma cells is due to constitutive activation of promoter elements that are also involved in the induction by IFNγ or results from a melanoma-specific activation pathway.

First of all, besides CIITA-PIII and CIITA-PIV-derived transcripts we also observed CIITA-PII-derived transcripts in both constitutive and IFNγ-induced HLA-DR expressing melanomas. It reveals transcriptional activation and promoter accessibility over a large section of the *MHC2TA* multipromoter region in melanomas. Interestingly, our investigations revealed that the constitutive activation of CIITA-PIII in melanomas and its induction by IFNγ is mediated through distinct regions. Furthermore, our results indicate that the *in vivo* constitutive activation of *MHC2TA* promoters in MHC class II molecule expressing melanoma cell lines is not mediated by constitutive activation of factors involved the IFNγ signaling pathway. Finally we observed a similar acetylation status of histones H3 and H4 at CIITA-PII, -PIII and -PIV chromatin in both melanoma cell lines that constitutively express CIITA and those that solely express CIITA following exposure to IFNγ. These observations are in support of the notion that expression of specific transcription factors determines *MHC2TA* promoter activation rather than lack of promoter accessibility in melanoma cells.

2. Materials and methods

2.1. Cell culture

The following cell lines were used in this study: HeLa (cervical carcinoma), THP-1 (monocytic leukemia), Ramos Ra.1 (EBV-negative Burkitt's lymphoma) and Jurkat clone E6-1 (acute T leukemia). The Mu89, Mu96, 136.2, MZMel3.0, 513D, 513E, 530C1, IGR39D, MA and EW melanoma cell lines employed in this study have been described previously (Versteeg et al., 1988; Ramirez-Montagut et al., 2000; Kurnick et al., 2001). All cell lines were cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% heat inactivated fetal bovine serum (Greiner, Alphen a/d Rijn, The Netherlands), 100 IU/ml streptomycin and 100 IU/ml penicillin. For IFNγ induction cells were treated with 500 U/ml of rIFNγ at the times indicated.

2.2. RT-PCR

Isolation of RNA and synthesis of cDNA was performed as described previously (van der Stoep et al., 2002a). CIITA products were amplified using the following primers specific for, respectively, CIITA-PI, CIITA-PII, CIITA-PIII, CIITA-PIV and CIITA-exon 2 (reverse); P1-sense (gb: AF000002) $5'$-$^{-452}$CTGCAGCCCCCAGCAGCCGAG^{472}-$3'$, P2-sense (gb: AF000005) $5'$-$^{-150}$TGGCGTCTGAGGCAACCACAAGC^{172}-$3'$ and CIITA-D-anti-sense (gb: HSCIITA) $5'$-$^{-428}$CATACTGGTCCAGTTCCGCGATATTGG^{402}-$3'$; primers P3 and P4 specific for CIITA-PIII and CIITA-PIV, respectively, have been