The p53-binding Protein MDM2 Gene Is Differentially Expressed in Human Breast Carcinoma

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Abstract

The human p53-binding protein murine double minute 2 (MDM2) is believed to function as a negative regulator of p53. The MDM2 gene was cloned and sequenced only recently and was found to be amplified in a variety of sarcomas. Although mutations in the p53 gene have been shown to occur in human breast carcinoma (HBC), no information is available on MDM2 gene expression in HBC. In this study we report for the first time that the MDM2 gene is differentially expressed in HBC. Our results demonstrate a correlation between the estrogen receptor (ER) status and the MDM2 mRNA levels. In contrast to the ER-negative cell lines, all the ER-positive cell lines were found to express higher levels of MDM2 mRNA. ER-positive ZR-75 cells express 30-fold higher levels of MDM2 mRNA than does the ER-negative cell line Hs578T. Estrogen enhanced albeit modestly the MDM2 mRNA levels in ER-positive MCF-7 cells. Estrogen enhancement of MDM2 mRNA levels was also observed in ER-negative MDA-MB-231 cells transfected with functional ERs. Our data thus suggest that estrogen may play an important role in HBC growth stimulation by modulating the expression of MDM2, which in turn may inactivate the p53 function.

Introduction

The human p53-binding protein MDM2 gene was cloned and sequenced only recently (1). The MDM2 gene encodes an mRNA of approximately 5.5 kilobases and the MDM2 gene itself was found to be amplified in a variety of sarcomas (1, 2). Originally isolated from murine fibroblast cells, MDM2 has been implicated in tumorigenicity (3, 4). It is now believed that the product of the MDM2 gene binds with the p53 protein and functionally inactivates it (1, 5). A wealth of information now suggests that, among the genetic abnormalities identified in wide array of tumors, mutations in the p53 gene are the most frequent (6–8). It is also believed that p53 regulates gene transcription by binding to DNA in a sequence-specific manner and thus abnormalities in p53 can result in aberrant regulation of genes by p53 (7). The identification of MDM2 as a negative regulator of p53 would suggest another potential mechanism for p53 inactivation in tumors in which p53 appears to be genetically intact (1, 5). Human breast carcinoma has been shown to harbor p53 mutations (Ref. 9 and references therein). In addition, a number of established human breast cancer cell lines have been shown to possess p53 mutations (Ref. 9 and references therein). The MDM2 gene expression in human breast carcinoma has not been previously investigated. This study was undertaken to investigate the expression pattern of the human MDM2 gene in breast carcinoma cell lines.

Materials and Methods

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Materials and Methods

Most of the materials used in this study have been described previously (10). E2 was obtained from Steraloids, Inc. (Wilton, NH). [32P]-dCTP (3000 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Cell Lines and Cell Culture. The MCF-7, ZR-75, and MDA-MB-231 cell lines were a generous gift of Dr. Marc Lippman (Lombardi Cancer Center, Washington, D.C.). The T47D, Hs578T cell lines were purchased from American Type Culture Collection (Rockville, MD). The cell culture conditions and preparation of charcoal-dextran and sulfatase-treated serum were as described previously (10).

DNA Probes and Southern and Northern Blot Analyses. The human MDM2 cDNA probe (1) was kindly provided by Drs. K. Kinzler and B. Vogelstein (Johns Hopkins University, Baltimore, MD). The human p52 (12) cDNA probe was purchased from American Type Culture Collection (Rockville, MD). Northern blot analysis was essentially as described (13). DNA blot analysis and genomic DNA extraction were performed according to standard procedures (14). Genomic DNA from HBC cell lines analyzed in this study and from normal human stomach mucosa was digested with EcoRI, and the blots were probed with human MDM2 cDNA probe.

Stable Transfections of ER-negative MDA-MB-231. ER-negative MDA-MB-231 cells plated at a density of 1 × 10⁶ cells/100-mm dish were cotransfected with 10 μg of expression vector pSG5-HEO (15) and 2 μg of the dominant selection vector pSV2neo (16) utilizing the calcium phosphate method as described in Sambrook et al. (14). The expression vector pSG5-HEO (kindly provided by Professor P. Chambon, Strasbourg-Cedex, France) carries the human ER coding region under the control of the SV40 promoter (15). Control cells were transfected with the pSV2neo vector alone. G418-resistant colonies were selected in medium containing 800 μg/ml G418 and were clonally expanded into mass culture. The ER-positive colonies were identified by Western immunoblotting utilizing monoclonal antibodies to human ER (kindly provided by Dr. G. L. Greene, University of Chicago) and colonies expressing ER levels comparable to the levels detected in MCF-7 cells were selected for further analysis.

Results and Discussion

Fig. 1 demonstrates the expression of the MDM2 gene in four ER-positive and three ER-negative HBC cell lines. In addition to an approximately 5.5-kilobase MDM2 mRNA as reported previously (1, 2), we also detected the expression of multiple bands in all the cell lines tested. Oliner et al. (1) have isolated multiple cDNAs for MDM2; whether the multiple bands for MDM2 noted in this study represent alternatively spliced forms of MDM2 mRNA or closely related genes remains to be elucidated. Fig. 1, however, clearly suggests that there is an apparent correlation between the expression of the MDM2 gene and the ER status of these cell lines. The MDM2 gene is expressed at a higher level in ER-positive cell lines than in the ER-negative cell lines. Quantitations of the 5.5-kilobase band representing MDM2 on Northern blots revealed that the ER-positive ZR-75 cell line expresses
the highest levels of *MDM2* mRNA among all the cell lines examined; there was an approximate 30-fold difference in the expression levels of *MDM2* mRNA noted in ZR-75 and the ER-negative Hs578T cells. Hs578T cells expressed the lowest levels of *MDM2* mRNA among all the cell lines tested. Despite the fact that the ER-positive T47D cells had the lowest *MDM2* mRNA levels among the ER-positive cell lines, they exhibited an approximate 2-fold higher (*n* = 3) *MDM2* mRNA levels than that noted in the ER-negative MDA-MB-231 cells. It is of note that T47D cells contain very low levels of ER and they also contain dominant negative mutant variants of ER (Ref. 17 and references therein). To further investigate whether the differential expression of *MDM2* mRNA as noted on Northern analysis was the result of gene amplification and/or rearrangements, Southern blot analysis was performed. The results obtained (data not shown) indicated that the high constitutive levels of *MDM2* mRNA noted in ER-positive cells was not the result of gene amplification as has been found in a number of sarcomas (1, 2). In addition, no gross alterations were observed in the *MDM2* gene in these cell lines when compared to DNA obtained from normal human stomach mucosa (data not shown). These findings clearly indicate that the differential expression of *MDM2* mRNA noted in HBC cells was the result of differential regulation of the *MDM2* gene at the transcriptional and/or at posttranscriptional level. Since there was an apparent correlation between the ER status and the levels of *MDM2* mRNA, we sought to investigate the regulation of *MDM2* gene by estrogen. MCF-7 cells are ER positive and have been studied extensively. These cells are growth stimulated by estrogen (18) and the regulation of a number of genes by estrogen has been investigated in these cells (Ref. 19 and references therein). MCF-7 cells were treated with a physiological concentration (1 nM) of E2 for different times and the Northern blot analyses revealed only a minimal effect of E2 on *MDM2* mRNA abundance (data not shown). Growth factor-mediated induction of growth related genes such as c-fos, c-jun, and c-myc has been demonstrated in several systems (20, 21). Serum modulation of *p33* at the mRNA and protein level has also been shown (Ref. 22 and references therein). *MDM2* is considered a negative regulator of *p53* and thus should play an important role in cell proliferation. In addition, we also found that the *t1/2* of *MDM2* mRNA is very short (40–45 min) in MCF-7 cells (data not shown) and is typical of the *t1/2* for the mRNAs encoded by immediate early genes. We, therefore, also investigated the effect of serum on the modulation of *MDM2* gene expression. MCF-7 cells were grown arrested by serum starvation and then treated with 15% fetal bovine serum for different periods of time. The results obtained (data not shown) demonstrated that the quiescent cells constitutively expressed *MDM2* mRNA and that serum stimulation of MCF-7 cells exhibited only a modest modulation of *MDM2* gene expression.

We have recently transfected ER-negative MDA-MB-231 cells with the expression plasmid pSG5-HEO encoding human ERs and characterized the clones that express functional ERs. We investigated whether the stable transfection of functional ERs modulate the *MDM2* gene expression in ER-transfected MDA-MB-231 cells. Fig. 2 demonstrates that E2 treatment of ER-transfected MDA-MB-231 cells (clone4) resulted in an approximately 3.2-fold increase (*n* = 2) in the steady-state *MDM2* mRNA levels. As expected, neither the parental nontransfected MDA-MB-231 cells nor the pSV2neo transfected cells responded to E2 treatment. E2 modulation of the known estrogen-regulated *p52* gene (12) expression was also investigated and as can be seen (Fig. 2), E2 increased the endogenous *p52* mRNA levels only in the ER-transfected MDA-MB-231 cells (Fig. 2), thus confirming the functionality of the transfected ERs. E2 modulation of *MDM2* mRNA

**Fig. 2.** Northern blot analysis of steady-state *MDM2* mRNA levels in ER-transfected and nontransfected MDA-MB-231 cells. Cells were grown in T75 flasks containing regular medium supplemented with 5% fetal bovine serum for 24 h, washed several times with 1 × phosphate-buffered saline, and then plated in phenol red-free medium supplemented with 5% charcoal-dextran and sulfatase-treated serum for another 48 h. Twenty-four h prior to E2 treatment cells were serum starved in phenol red-free medium and then treated with 1 nM E2 for 48 h. E2 treatment was in phenol red-free medium supplemented with 5% charcoal-dextran and sulfatase-treated serum. Total cellular RNA (35 µg/lane) was analyzed by Northern hybridization and the same blots were also probed with *p52* cDNA. Ethidium bromide staining of the gels (data not shown) revealed RNA integrity and comparable loading. Results of 2 independent experiments (representing 2 separate treatments and RNA preparations) for ER-transfected MDA-MB-231 cells are shown.

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This suggests that the E2 effect on MDM2 mRNA levels appears in terms of the ER levels, growth behavior, and cell morphology. Similar type or pSV2neo transfected MDA-MB-231 cells (Fig. 2; data not shown). Increase in the steady-state MDM2 mRNA levels (data not shown). CloneS exhibits characteristics similar to clone4 in breast tumor progression p53 may still be intact but is negatively controlled only to the cell lines analyzed is not clear at this time. More cell lines and tumor samples are required to further investigate and firmly establish this correlation. The data presented in this study, however, suggest that the correlation observed may not be coincidental. For example, estrogen enhanced the A/DA/2 mRNA levels in the ER-positive breast tumor cells. Somatic Cell Mol. Genet., 13: 235-244, 1987.

References
11. Lamph, W. W., Wamsley, P., Sassone-Corsi, P. and Verma, I. M. Induction of proto-oncogene (25, 26). It is thus tempting to speculate that at an early stage in tumor progression the more aggressive ER-negative Hs578T cell line. Whether the correlation between the ER status and the levels of MDM2 mRNA is coincidental and re...
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