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-negative 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

Materials. Most of the materials used in this study have been described previously (10). E2 was obtained from Steraloids, Inc. (Wilton, NH). [3H]-dCTP (300 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 retinoic acid-resistant RRO-I cell line was developed in our laboratory as previously described (11). The MDA-MB-468 cell line was kindly provided by Dr. Anne Hamburger (University of Maryland, Baltimore, MD). 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). RNA extraction and Northern blot analysis were essentially as described (13). Southern 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 x 10^5 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 abbreviations used are: MDM2, murine double minute 2; HBC, human breast carcinoma; ER, estrogen receptor; E2, 17β-estradiol; cDNA, complementary DNA; t1/2, half life.
cDNA. Ethidium bromide staining of the gels (data not shown) revealed RNA integrity and nontransfected MDA-MB-231 cells. Cells were grown in T75 flasks containing media and RNA preparations) for ER-transfected MDA-MB-231 cells are shown. and equal loading. Results of 2 independent experiments (representing 2 separate treat was analyzed by Northern hybridization and the same blots were also probed with pS2 with 5% charcoal-dextran and sulfatase-treated serum. Total cellular RNA (35 ¿g/lane) treated with I nM E2 for 48 h. E2 treatment was in phenol red-free medium supplemented with I X phosphate-buffered saline, and then plated in phenol red-free medium supple

Fig. 1. Northern blot analysis of MDM2 mRNA expression in ER-positive and ER-

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 X phosphate-buffered saline, and then plated in phenol red-free medium supple

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.4 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 pS2 gene (12) expression was also investigated and as can be seen (Fig. 2), E2 increased the endogenous pS2 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 was further investigated in another ER-transfected MDA-MB-231 clone (clone5). Clone5 exhibits characteristics similar to clone4 in terms of the ER levels, growth behavior, and cell morphology. Similar treatment of clone5 with E2 also resulted in an approximately 3-fold increase in the steady-state MDM2 mRNA levels (data not shown). This suggests that the E2 effect on MDM2 mRNA levels appears specific and was due to the presence of functional ERs in the MDA-MB-231 cells since E2 did not modulate MDM2 mRNA in the wild-type or pSV2neo transfected MDA-MB-231 cells (Fig. 2; data not shown).

This is the first report that has investigated the expression of the MDM2 gene in HBC. In this report we have demonstrated that the MDM2 gene is differentially expressed in HBC cells and there appears to be a correlation between the ER status and the levels of MDM2 mRNA. MDM2 mRNA levels were approximately 30-fold higher in ER-positive ZR-75 cells when compared with the levels found in the ER-negative Hs578T cell line. Whether the correlation between the ER status and the levels of MDM2 mRNA is coincidental and restricted 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-transfected MDA-MB-231 cell line. The mechanism by which estrogen modulate MDM2 mRNA accumulation remains to be determined. We found that the MDM2 mRNA is very labile with a t1/2 of 40-45 min which is in close agreement with recently reported 30-min t1/2 of MDM2 protein (23). The estrogen effect on MDM2 mRNA levels in MCF-7 cells was, however, modest and the biological significance of this increase in MDM2 mRNA must await further study. Estrogen is a strong stimulator of HBC growth (18). ER-positive HBC cells are thought to represent an earlier stage in tumor progression than the more aggressive ER-negative cells (24, 25). The hormone-independent ER-negative HBC cells are mostly undifferentiated and have a high proliferative index (25). The basis of their more aggressive phenotype is not clear but is thought to be due to higher constitutive levels of secreted growth factors and the up-regulation of oncopgenes (25, 26). It is thus tempting to speculate that at an early stage in breast tumor progression p53 may still be intact but is negatively regulated by higher levels of MDM2 which is under hormonal control. Because of selective pressure towards a more aggressive phenotype, the cells tend to lose ERs and during this transition, they may also lose control of MDM2 expression and accumulate mutations in p53 gene. Consistent with this hypothesis would be a recent report (9) that studied a number of established and primary HBC cell lines and found that the primary tumors exhibited a lower incidence of p53 mutations. It was thus concluded that the occurrence of p53 mutations in HBC is a late event and often affects only a subset of tumors (9). Furthermore, the ER-positive MCF-7 and ZR-75 cells which we found (in this study) to express higher levels of MDM2 mRNA appear to contain wild-type p53 (9). A recent report by Allred et al. (27) studying 700 patients actually found a significantly higher incidence of p53 mutations in ER-negative breast tumors. This may also further support the notion that the ER-positive cells that have a lower incidence of p53 mutations may indeed have higher expression levels of MDM2. The study conducted by Allred et al. (27) also found that a number of tumors showed normal p53 sequence yet they expressed higher levels of p53. Wild-type p53 is very labile protein and has a very short t1/2 (Ref. 27 and references therein). Mutant p53 in contrast tends to accumulate in tumors as a more stable form (Ref. 27 and references therein). It may thus be possible that in tumors which exhibit the wild-type p53 sequence, the protein itself is stabilized and inactivated after its association with MDM2. Further studies are obviously indicated to investigate in detail the status of MDM2 in HBC.

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References
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