Altered Regulation of Cyclin G in Human Breast Cancer and Its Specific Localization at Replication Foci in Response to DNA Damage in p53+/+ Cells*

(Received for publication, December 3, 1998)

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Cyclin G, a recent addition to the cyclin family, was initially identified in screens for new src kinase family members and soon thereafter by differential screening for transcriptional targets of the tumor suppressor gene, p53. We have identified cyclin G as being overexpressed in breast and prostate cancer cells using differential display polymerase chain reaction screening. We demonstrate here that cyclin G is overexpressed in human breast and prostate cancer cells and in cancer cells in situ from tumor specimens. Cyclin G expression was tightly regulated throughout the cell cycle in normal breast cells, peaking at the S and G2/M phases of the cell cycle with lower levels in G1. The cell cycle-dependent expression was absent in breast cancer cells. Following DNA damage in normal p53+/+ cells, cyclin G is triggered to cluster in discrete nuclear DNA replication foci that contain replication-associated proteins such as proliferating cell nuclear antigen (PCNA). While p53+/− cells displayed a faint cyclin G nuclear staining pattern, there was no increased expression and no change in distribution of the staining pattern after DNA damage. The specific subcellular localization of cyclin G at DNA replication foci provides an additional link between p53-mediated growth arrest and cell cycle regulation and suggests that cyclin G may act as an effector of p53-mediated events by functional association with replication foci protein(s).

Human cancer development is a multistage process that results from the stepwise acquisition of genetic alterations. These alterations may involve the dysregulation of a variety of normal cellular functions, leading to the initiation and progression of a tumor. Among normal cellular functions, regulatory control of the cell cycle plays an important role in normal cell proliferation, and genetic alterations that affect cell cycle control have been shown to be associated with tumor progression (reviewed in Refs. 1–3). Cyclins are prime cell cycle regulators and control the major check points in cell cycle transitions of eukaryotic cells (2). In association with a family of cyclin-dependent protein kinases, cyclins maintain the orderly progression of cells through the various phases of the cell cycle. The link between oncogenesis and cyclins has been made with the aberrant expression of two cyclins (cyclin A and D1) in human tumors (2, 4–6). Other members of the cyclins (i.e. cyclin B, D3, and E) have also been shown to have altered expressions in human tumors, including breast and prostate cancers (7–10).

Cyclin G (Cyc G), a recent addition to the cyclin family, was initially identified in screens for the src kinase family in rat fibroblasts and soon thereafter by differential screening for transcriptional targets of the tumor suppressor gene p53 (11, 12). Cyc G has homology to fission yeast Cig1, B-type cyclins, and human cyclins A and I (13). Unlike other cyclin family members, Cyc G lacks a “destruction box” motif that controls the ubiquitin-dependent degradation but contains an epidermal growth factor receptor-like autophosphorylation motif (11, 13). Cyc G is the only known cyclin that is transcriptionally activated by the p53 tumor suppressor gene, suggesting that it may play a role in p53-mediated cell growth control (12–16). Furthermore, despite its high homology with other cyclins, Cyc G has not yet been matched with a cyclin-dependent kinase binding partner, and its biological function remains elusive. However, Cyc G forms a complex with B regulatory subunits of protein phosphatase 2A following its induction by p53 (17). In contrast to most p53 target genes such as p21/Waf1/Cip1/Sdi1, Bax1, IGF-BP3, and Gadd45, Cyc G does not seem to exert a tumor-suppressive role but rather, like other cyclins or proto-oncogenes, plays a growth-promoting role (15, 18–20). Consistent with this observation, Cyc G overexpression has been observed in human osteosarcoma cells (18, 19).

Initially, we identified the cyclin G gene using differential screening (differential display PCR) (21) between human normal and tumor breast cells. Here we report the expression pattern, cellular localization, cell cycle regulation, and Cyc G response to p53 induction in normal and breast cancer cells. We show that aberrant expression of Cyc G may be closely associated with the tumorigenic process. Our demonstration that following DNA damage, Cyc G is triggered to cluster in highly organized nuclear compartments representing DNA replication foci may provide an additional link between p53-mediated growth arrest and cell cycle control. Together, these data sug-

* This work was supported by National Institutes of Health grants and a grant from the Massachusetts Department of Public Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: Cyc G, cyclin G; NMEC, normal mammary epithelial cell; hNMEC, human normal mammary epithelial cell; mNMEC, mouse normal mammary epithelial cell; PACS, fluorescence-activated cell sorter; MMC, mitomycin C; PCR, polymerase chain reaction; FBS, fetal bovine serum; DMEM, Dulbecco’s modified Eagle’s medium; ABC, avidin-biotin peroxidase complex; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen.
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MATERIALS AND METHODS

**Differential Display PCR and cDNA Library Screening**—The quality of total RNA was tested by Northern blot analysis before reverse transcription. Differential display PCR was performed using the RNAImage™ Kit 1 according to the manufacturer’s protocol (GenHunter). The cDNA products were amplified by the polymerase chain reaction using H-AP3 5’ primer (5’-AACCTGGTCGTCGAC-3’) and H-TG3’ primer. The band of interest was isolated, PCR-reamplified using the same primers as above, cloned into pGEM-T vector (Promega), and sequenced by the dideoxynucleotide chain termination method with Sequenase (U.S. Biochemical Corp.). This partial-length clone was labeled with α-32P-dCTP using the random primed synthesis method and used as a probe to obtain the full-length clone by screening a cDNA library prepared from normal mammary epithelial cells (22).

**Cell Lines and Cultures**—Primary human normal mammary epithelial cells (hNMECs) were established from reduction mammoplasties obtained through the Cooperative Human Tissue Network and designated 14N and 15N, as described (23–25). Mouse mammary epithelial cells (mNMECs) were derived from 129/Sv (p53+/−) mice obtained from Jackson Laboratories. These cells were grown in DFCT-1 medium (D Complete) as described (24) and were used at early to mid-passage, i.e., 5–10 population doublings.

Human breast cancer cells (MCF7, T47D, HS578T, MDAMB435, and MDAMB468) were obtained from ATCC and maintained in DMEM, 10% FBS. Primary human normal prostate cells were derived from normal adjacent tumor tissue biopsies received from the Cooperative Human Tissue Network and designated NPrEC-1 and -2. These cultures were grown in Clonetop Epithelial Media. The human prostate carcinoma cell lines PC3, LNCaP, and DU145 were purchased from the ATCC and maintained in DMEM, 10% fetal bovine serum (FBS). Primary human normal prostate cells (hNMEC) was DMEM, 10% fetal bovine serum (FBS). Primary human normal prostate cells were derived from normal adjacent tumor tissue biopsies received from the Cooperative Human Tissue Network and designated NPrEC-1 and -2. These cultures were grown in Clonetop Epithelial Media. The human prostate carcinoma cell lines PC3, LNCaP, and DU145 were purchased from the ATCC and main-

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**Results**

**Identification of the Cyc G Gene as a Breast Tumor-specific Gene Through a Differential Display Method**—Using the differential display PCR technique (21) to survey differentially expressed clones in human mammary normal and tumor cells, we isolated a cDNA fragment, 15-1 clone, with higher abundance in cancer (T47D and MDAMB435) compared with normal cells (14N and 15N). After reamplification and subcloning, 15-1 cDNA fragment was used to confirm the differential display patterns on Northern blot. Southern and hybridization analysis of cloning 15-1 clone to those within the GenBank™ data base revealed a 100% sequence match to the 3′-untranslated region of the human cyc G cDNA (13, 14). Using this partial clone as a probe, a normal human mammary cDNA library was screened and a full-length cDNA was isolated.

**Overexpression of Cyc G in Breast and Prostate Cancer**
Altered Regulation of Cyclin G in Human Cancer

Fig. 1. Overexpression of cyclin G in human mammary and prostate carcinoma cell lines. Western blot analysis was used to determine the level of Cyclin G protein in normal cells derived from hNMECs (cell strains 14N (primary cultures) and 15N (passage 7 cultures)) and in several mammary epithelial tumor cell lines MCF7, T47D, Hs578T, MDAMB435, and MDAMB436. Cyclin G protein expression was also measured in normal primary (NPrEC-1 and -2) and tumor prostate cell lines LNCaP, DU145, PC3, and NDI. Alliquots of cell lysates derived from the indicated cell lines were loaded in each lane and then separated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membrane and then immunoblotted with antibodies Cyclin G and β-actin, respectively. Immunoblotting for β-actin was used for equal loading of protein samples.

Cells—We first examined the expression of Cyclin G in human mammary normal and tumor epithelial cells. As shown in Fig. 1, all human breast cancer cell lines examined had higher levels of Cyclin G protein expression, regardless of estrogen receptor status, but a low level of expression was detected in human normal mammary epithelial cells (14N and 15N). In addition, higher levels of Cyclin G protein were observed in human prostate tumor cell lines when compared with normal prostate epithelial cells (Fig. 1).

The distribution of Cyclin G protein was determined using immunohistochemistry in a number of human normal and tumor breast tissue specimens to define the relationship between Cyclin G expression and breast cancer development. A total of 40 different cases were analyzed, including 10 normal biopsies, and 30 tumor biopsies (10 in situ carcinomas, seven infiltrating ductal carcinomas, six infiltrating lobular carcinomas, and seven fibroadenomas). Among the normal biopsies, little or no nuclear or cytoplasmic Cyclin G staining was observed either in the epithelial or stromal cells of the ductal units (Fig. 2A). However, 22 of the 30 tumor samples showed increased Cyclin G expression that was specific to nuclei of epithelial cells (Fig. 2, C, E, and G). Positive nuclear staining was observed in seven of the 10 in situ carcinomas, five of six infiltrating ductal carcinomas, four of six infiltrating lobular carcinomas, and six of seven fibroadenomas. With the exception of the fibroadenomas, stromal cells of the tumor biopsies showed no staining. Our findings indicate that Cyclin G overexpression is a frequent occurrence in breast cancer.

Cell Cycle-dependent Expression of Cyclin G in Normal Versus Tumor Mammary Epithelial Cells—To gain further insight into the possible function(s) of Cyclin G, we compared the expression patterns of Cyclin G mRNA and protein throughout the cell cycle in normal versus tumor cells (Fig. 3, A and B). A normal human breast epithelial cell strain (15N) and a human mammary cancer cell line (MCF7) were synchronized by three different methods: 1) lovastatin (cells arrest at G1); 2) growth factor deprivation (G0; normal cells only); and 3) nacodazole (G2/M). Synchronization of both cell types was monitored by [3H]thymidine incorporation, histone H4 expression, and flow cytometry analysis (FACS) (Fig. 3C). Northern and Western blot analyses of cells harvested at regular intervals revealed a differential expression pattern of Cyclin G during cell cycle progression in normal versus tumor cells (Fig. 3). In normal cells (NMECs), Cyclin G mRNA and protein levels were low to undetectable during most of G1 phase. As cells entered into S phase, a marked induction of Cyclin G was observed, which remained high throughout the S and G2/M phases, correlating with histone H4 expression (Fig. 3A). A similar pattern of expression was observed with all three synchronization methods in normal cells. However, in a human breast cancer line, MCF7, cell cycle-dependent expression was absent; instead, there was a consistent high level of expression throughout the cell cycle (Fig. 3B). MCF7 cells were synchronized by lovastatin or nacodazole, both of which gave similar patterns of Cyclin G expression (Fig. 3B). Cyclin G overexpression in cancer cells may result from an altered cell cycle regulation.

Differential Induction of Cyclin G Expression by p53—Cyclin G is a transcriptional target gene of p53 that contains two p53-binding elements in its promoter region (12, 16). High basal levels of Cyclin G expression in tumor cells lacking functional p53 suggest that altered expression of Cyclin G may be independent of p53 dysregulation and may contribute to the malignant phenotype of cancer cells. To examine the possibility of an altered response of Cyclin G by p53 in some cell types, we have utilized a tetracycline-regulated p53-inducible system in tumor cell lines (Sao2-p53 and EJ-p53) that contain nonfunctional p53 and a high basal level of Cyclin G. As shown in Fig. 4A, after tetracycline removal, p53 mRNA was readily detectable (data not shown) as well as p21 mRNA, a p53 transcriptional target gene. After removal of tetracycline in both cell lines, Cyclin G levels remained unchanged, although p21 mRNA was induced dramatically after 12 h, indicating p53 transcriptional activity. This level of expression remained consistent and unchanged over time (up to 5 days), suggesting no specific induction of Cyclin G expression by p53 in these cell types. However, the expression of Cyclin G was dramatically induced following wild-type p53 activation in VhD and Vm10 cells (27), which express low basal levels of Cyclin G (Fig. 4B). The VhD and Vm10 are immortalized mouse embryo fibroblast cell lines containing a temperature-sensitive p53 mutant (Val to Ala mutation at codon 135, tsp53), which express a nonfunctional p53 at 37–39 °C and fully functional p53 at 32 °C. The VhD cells undergo reversible G1 arrest in a p53-dependent manner at 32 °C, while the Vm10 cell line, which constitutively expresses tsp53 and the c-myc oncogene undergoes apoptosis at 32 °C (27). The expression of p21 in these two tsp53 cell lines was low before the temperature shift and increased with similar kinetics to Cyclin G induction. Altogether, these data demonstrate that the activation of Cyclin G expression by p53 induction or activation is universal for both growth arrest and apoptotic pathways but only observed in cells containing low basal expression of Cyclin G.

Localization of Cyclin G to Nuclear Replication Foci in Response to DNA Damage—In order to determine regulation and localization of Cyclin G in response to DNA damage, we first examined the expression and localization of Cyclin G in mNMECs isolated from p53-deficient mice after treatment with DNA damaging agents. Cells from the mammary glands of p53 null mice as well as cells from wild type mice were treated with actinomycin D (not shown) or MMC. Northern blot analysis showed that Cyclin G mRNA expression was induced after treatment with actinomycin D in wild-type cells, while in p53 null cells, Cyclin G mRNA was hardly detectable, and no induction was observed after DNA damage treatment (Fig. 5A, top). We also examined the cells for distribution of Cyclin G protein before and after DNA damage with MMC using immunofluorescence staining (Fig. 5A, bottom). In wild-type cells, the staining with anti-Cyclin G antibodies revealed an amplification of distinct punctate patterns in nuclei following DNA damage treatment, while untreated cells had a lower number of punctate nuclear signals. These MMC-treated wild-type cells also showed strong recruitment of p53 to the nuclei. However, p53 null cells displayed a very faint Cyclin G nuclear staining pattern, and there was no increase of signals after DNA damage (Fig. 5A).
We next compared the distribution of Cyc G before and after DNA damage in human normal and tumor mammary epithelial cells (Fig. 5B). When 15N cells (hNMEC) were methanol-fixed and stained for Cyc G, a distinct but low level nuclear signal was detected (Fig. 5B), similar to that seen in p53+/+ mNMEC (Fig. 5A). The staining correlated well with the Western blot analysis, which showed low levels of Cyc G protein in NMECs compared with tumor cell lines (Fig. 1). After exposure to MMC, a dramatic amplification of Cyc G signal was observed, which was localized to the nucleus in a discrete staining pattern, consisting of 6–10 brightly labeled clusters per cell reminiscent of DNA replication foci (Fig. 5B). In human mammary cancer cells, the patterns of Cyc G localization were not as distinct as those seen in normal cells, although the staining was still present in the nucleus (Fig. 5B). We used two tumor mammary cancer cell lines: MCF7, containing wild-type p53, and T47D, containing a nonfunctional mutated p53 gene (30). Both lines have higher basal expression levels of Cyc G as compared with hNMEC (Fig. 5B). MCF7 cells displayed amplified nuclear staining signals in response to DNA damage, but T47D cells did not show any induction of Cyc G protein by DNA damage (Fig. 5B). The distribution of staining for both cell types also appeared predominantly nuclear.

The localization observed after DNA damage in normal cells suggests that Cyc G may be recruited to areas of DNA replication and repair, delineated as DNA replication foci, where proteins such as PCNA and several cyclins including cyclin A and D have been shown to colocalize (31). Given the pattern of Cyc G localization in the nucleus and the potential involvement of Cyc G in DNA replication and repair, we explored the possibility of an association between Cyc G and PCNA, which plays an essential role in nucleic acid metabolism as a component of the replication and repair machinery. Co-localization between Cyc G and PCNA proteins was tested on MMC-treated...
(10 μg/ml for 12 h) 15N cells using two-color immunostaining (Fig. 6). Detergent extraction of the cells followed by methanol fixation removes detergent-soluble PCNA, and enables the clear visualization of detergent-insoluble PCNA, the form that is tightly complexed to DNA in replication foci (31–34). A colocalization of PCNA and Cyc G was observed in defined foci in the nuclei of DNA-damaged cells. To assess whether a PCNA/Cyc G co-localization pattern was indicative of an association between the two proteins, immunoprecipitation analyses were also carried out. However, we failed to detect a co-immunoprecipitation complex between these two proteins, raising the possibility that Cyc G may function at the site of DNA replication but does not directly interact with PCNA.

DISCUSSION

Accumulating evidence suggests that altered expression of cyclins, including cyclins A, D1, and E, may contribute to the malignant phenotype of human cancers such as mammary and prostate cancers (5–10). Ongoing efforts in the discovery of candidate genes whose function or expression is altered during the course of breast carcinogenesis led to the identification of Cyc G whose expression was up-regulated in breast and prostate cancer cells both in vitro and in vivo. In an attempt to understand the potential role of Cyc G in the process of tumorgenesis, we have studied its expression pattern, cell cycle regulation, response to DNA damage, and subcellular distribution in normal and cancerous cells.

To assess the in vivo relevance of Cyc G overexpression in breast and prostate cancer cell lines, we examined its expression pattern in clinical breast cancer specimens as well as normal breast tissues by immunohistochemistry. Consistent with the high levels of expression observed in cell lines, a significant Cyc G overexpression occurs in the majority of in situ and invasive ductal and lobular carcinomas examined. Cyc G staining is restricted to the nuclei in the malignant tumor biopsies, whereas staining in normal ductal units is barely...
Altered Regulation of Cyclin G in Human Cancer

and has potential diagnostic/prognostic value in cancer development. Others have reported the overexpression of Cyclin G in osteosarcoma cells (19, 20) and reported that antisense Cyclin G expression inhibited growth of human osteogenic sarcoma cells in vitro and in a nude mouse in vivo tumor model and resulted in a significant increase in the incidence of apoptosis (19, 35). In addition, ectopic overexpression of Cyclin G in human RKO colon carcinoma cell line accelerated cell growth, and the transfection of normal human fibroblasts with Cyclin G increased their proliferative lifespan (15). These data, as well as our findings, suggest that Cyclin G may possess or correlate with oncogenic potential. However, Cyclin G has also been identified as a transcriptional target gene for the tumor suppressor gene p53, and its promoter sequence contains functional p53 binding sites that efficiently respond to p53 (12, 16). In contrast to other p53-regulated genes, Cyclin G appears to behave much like other cyclins or protooncogenes. Although several possible roles of Cyclin G have been speculated in a p53-mediated tumor suppression, the function of Cyclin G remains elusive, and its classification as a cyclin rests solely on sequence similarities with other members of the cyclin family.

It has been demonstrated that Cyclin G is constantly expressed throughout the cell cycle without any specific pattern in HeLa cells (11, 12, 14). In other studies in which they examined Cyclin G expression during the cell cycle in tumor cell lines such as murine B cell lines and NRK-49F cells, there was no change in Cyclin G expression levels throughout the cell cycle (11, 13, 14). Consistent with the previous data, we have found that Cyclin G is constitutively expressed in two breast cancer cell lines in all phases of the cell cycle. However, like other members of the cyclin family, Cyclin G expression is gradually increased with cell cycle progression, peaking in late S and G2/M phases in human normal mammary epithelial cells. Thus, constitutive Cyclin G expression during the cell cycle progression may contribute to the dysfunction of cell cycle control in transformed cells, which is tightly regulated in normal cells.

We have observed that expression and induction of Cyclin G parallels that of p53 in human NMECs after DNA damage. Although Cyclin G is a transcriptional target gene of p53, its overexpression in the majority of breast and prostate cancer cells does not simply reflect the p53 status in those cells; i.e. whether there exists wild type p53 in cells (MCF7) or a mutated form (T47D), Cyclin G is expressed at higher than normal levels. This may be due, in part, to other mechanisms that regulate Cyclin G expression independent of p53. It is clear that ectopic wild-type p53 expression in p53 null tumor cells can transactivate expression of p53 target genes by several fold (36, 37). Our studies utilizing a tetracycline-regulated p53 inducible system in two p53-null tumor cell lines (Saos2, EJ) demonstrate that Cyclin G levels remain unchanged following p53 overexpression, although other p53-inducible genes such as p21 and mdm2 were dramatically induced in a short time. Despite persistent ectopic p53 expression and transcriptional activity, the level of Cyclin G expression remained consistent and unchanged for up to 5 days in both EJ and Saos2 cells. However, the expression of Cyclin G was dramatically induced following wild-type p53 activation in VhD and Vm10 cells, which express low basal levels of Cyclin G (Fig. 4B). The VhD and Vm10 cells are immortalized mouse embryo fibroblast cell lines that contain a temperature-sensitive p53 mutant (tsp53), but they do not appear to assume malignant phenotypes (27, 38). Upon a shift to the permissive temperature and p53 activation, VhD cells become arrested in G1, whereas Vm10 cells undergo apoptosis (39). These data indicate that when the basal expression levels are high, Cyclin G becomes unresponsive to p53 transcriptional activation. They also demonstrate that, at least in artificial

![FIG. 5. Cellular localization of Cyclin G and its response to DNA damage. A, top, p53-dependent Cyclin G induction following mitomycin C treatment (10 μg/ml) in p53+/+ versus p53−/− mouse normal mammary epithelial cells (mNMECs). p53−/− or +/+ mNMECs were treated with/without DNA damage agent, MMC, for 24 h, and total RNA was isolated for Northern blot analysis. The Northern blot was consecutively hybridized with Cyclin G, p21, and 36B4 probes. 36B4 was used a loading control. Bottom, cellular localization of Cyclin G with/without MMC in p53+/+ and p53−/− mNMECs. Cells were incubated MMC (10 μg/ml) and fixed in methanol and immunostained for Cyclin G antibodies for cellular localization. Notice that the staining was restricted to the nucleus and was in a characteristic pattern, consisting of 5–9 distinct clusters in each cell in 15N cells. In two mammary tumor cell lines, MCF7 and T47D, Cyclin G staining also appeared predominantly nuclear, although the discrete clusters of labeling were not as pronounced, as was the case in normal cells (15N). Instead, the punctate staining in the tumor cells appeared to be distributed throughout the nucleus with little overt spatial organization. No major increase of Cyclin G labeling was observed following DNA damage. Bar, 10 μm.](image-url)
systems where p53 is ectopically expressed, the expression of Cyc G is increased irrespective of subsequent cell fate, whether it is growth arrest or apoptosis. Nonetheless, the Cyc G response to p53 is only seen in cells containing the low basal expression of Cyc G (e.g., normal cells) or in nonmalignant cells. Although the mechanisms responsible for this differential response of Cyc G induction by p53 remain to be elucidated, one possibility is that Cyc G mRNA might be stabilized in tumor cells and that high basal levels of Cyc G expression may act in a negative feedback loop, suppressing p53-mediated Cyc G transcription. Another possibility is that in tumor cells, Cyc G is being transcriptionally independent of p53 transcriptional activity at a maximal rate with no further increase in transcription upon p53 induction/activation. Therefore, the constitutive expression of Cyc G may account for dysregulation of the normal cell cycle in cancer cells, hence affecting their response to various stimuli such as DNA damage.

It is known that in the mammalian nucleus, DNA is replicated in discrete nuclear foci and that PCNA, a replication-associated protein, specifically localizes at these replication foci. Previously, a direct link between cell cycle regulation and DNA replication was established when it was shown that Cyc A and cyclin-dependent kinase 2, a complex needed for entry into S phase, specifically localize at nuclear replication sites. Given that Cyc G is dramatically recruited to replication foci following DNA damage in normal cells, it is likely that Cyc G acts as an effector in p53-mediated events by functional association with one or more of the replication foci proteins. Our attempts at elucidating one such association, namely, Cyc G and PCNA, by co-immunoprecipitation did not reveal any physical association between these two proteins. Nonetheless, the subcellular colocalization of Cyc G at DNA replication foci suggests a role for Cyc G in regulating DNA replication. The demonstration of colocalization by itself does not prove Cyc G’s involvement in DNA replication but is consistent with the speculation that in association with replication protein(s), Cyc G may act as an important regulator of cell proliferation and response to DNA damage in normal cells. Recent experiments have demonstrated that Cyc G-immunoprecipitated complexes collected from Cyc G transfectants displayed an appreciable level of cyclin-dependent kinase activity and contained the retinoblastoma protein (15), thus suggesting potential involvement of Cyc G during the cell cycle progression. Further studies are needed to identify Cyc G-associated proteins and their relevance to its potential growth arrest function.

Cultured cells from Ataxia-Telangiectasia patients (A-T cells) are unable to arrest at the G1-S boundary following DNA damage such as irradiation. A recent report suggested that Cyc G may contribute to G2/M arrest of cells in response to DNA damage (40). From our initial efforts to define whether Cyc G responds similarly to p53 and its target gene p21 following DNA damage in A-T cells, five out of six AT cell lines revealed an aberrant Cyc G response following DNA damage treatment with actinomycin D (10 ng/ml) when compared with normal dermal fibroblasts, while these five A-T cell lines as well as the normal dermal fibroblasts showed an increase of p53 protein and p21 expression. Thus, altered response of Cyc G by DNA damage in A-T cells may contribute to the DNA repair defect of these A-T cells.

The present study demonstrates that Cyc G is overexpressed in breast cancer tissues and cell lines, independent of p53 status. Although the Cyc G promoter contains two p53 response elements, the transcriptional regulation of Cyc G by p53 is highly dependent on the cellular context in which p53 is activated. Our data demonstrate that Cyc G is expressed with periodicity throughout the cell cycle of normal cells, but not in tumor cell lines, and that localization of Cyc G at replication foci is quite distinct and may provide a clue for involvement in DNA replication. Finally, overexpression of Cyc G in human cancers such as breast and prostate may prove to be an important prognostic marker for tumor initiation and/or progression. Future studies should be directed at testing the diagnostic value of Cyc G on a larger scale to firmly establish a correlation between the degree of Cyc G expression and breast or prostate cancer progression. It is also conceivable that new therapeutic approaches directed specifically at down-regulating Cyc G in breast and prostate cancers might cause a reduction of tumorigenic potential.

Acknowledgments—We are grateful to Dr. X. Wu for the tsp-p53 cell lines and Dr. P. Narayan for the ND1 cell line. We thank K. Claffey for useful suggestions and D. Campbell and M. Tang for technical help.

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