ANTICANCER RESEARCH *25*: 273-282 (2005)

Cyclin D1 Overexpression is Associated with Estrogen Receptor Expression in Caucasian but not African-American Breast Cancer

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Abstract. *Background: African-American (AA) breast cancer patients consistently show a shortened survival when compared with Caucasian patients. This worse prognosis is most likely due to a combination of socioeconomic factors and differences in tumor biology. Previous studies have demonstrated that cyclin D1 overexpression is strongly associated with estrogen receptor (ER) expression in breast cancer, but these series either included primarily Caucasian patients or did not specify race. Materials and Methods: We analyzed 200 breast cancer cases obtained from AA and Caucasian patients who were matched on age, stage, ER status, and year of diagnosis. We examined expression levels of cyclin D1, p53, p27^{Kip1} (p27), and p21^{Cip1} (p21), and correlated their expression with ER status. Results: Cyclin D1, p53, p27, and p21 expression rates were similar in matched cases of AA and Caucasian breast cancer (p values>0.05). However, cyclin D1 overexpression was significantly associated with ER status in only the Caucasian (p=0.0005), and not the AA cases (p=0.07). Conclusion: This finding suggests a novel biological difference, which may contribute to the more aggressive phenotype of AA breast cancer.*

Abbreviations: AA, African-American; ER, estrogen receptor; p27, p27^{Kip1}; p21, p21^{Cip1}; NYPH, New York Presbyterian Hospital; AJCC, American Joint Committee on Cancer; IRB, Institutional Review Board; PR, progesterone receptor; IHC, immunohistochemistry; DAB, diaminobenzidine; MIB-1, proliferation index; OR, odds ratio; CI, confidence interval; HR, hazard ratio.

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Key Words: Cyclin D1, breast cancer, African-American, estrogen receptor.

Despite an estimated thirteen percent lower incidence of breast cancer among African-American (AA) women compared with Caucasian women, breast cancer mortality rates remain 28% higher among AAs (1). This difference most probably results from a combination of both socioeconomic and biological factors (for review, see (2)). Breast cancers that develop in AA women are more frequently of higher grade and less often estrogen receptor (ER)-positive, when compared with those that develop in Caucasian women (3). Breast cancer also develops more frequently in younger AA women, *i.e.*, those in the 30-49 year age group (2, 4). Previous reports of AA breast cancer have uncovered unique mutations in the brca1 and brca2 breast cancer susceptibility genes (5-8), unique "aggressive" p53 gene mutations (9), and polymorphisms in the ras gene (10). Although these genetic differences may help to explain the aggressive behavior of AA breast cancer, they do not demonstrate actual differences in tumor biology at the protein level. Therefore, these differences do not suggest specific clinical targets for which specific therapies can be developed for the AA breast cancer population. Furthermore, previous comparisons of cases of breast cancer in Caucasian and AA patients have failed to demonstrate any differences in the frequency of expression of other potentially useful clinical markers of breast cancer, including Her-2/neu, Ki-67, EGFR, CEA, cathepsin D, or pS2 (11-13). Some of these reports may have lacked the statistical power to detect any significant differences.

We recently reported that cyclin D1 overexpression appeared to be more prevalent in cases of female breast cancer obtained from non-Caucasian patients when compared with those from Caucasian patients (14). In our series, the frequency of cyclin D1 overexpression in cases of breast cancer from AA, Hispanic, and Asian patients ranged from 74% to 80% of the samples. This was in contrast to a value of 59% (p=0.051) for the cases obtained from

Caucasian patients. When cases from each of the three non-Caucasian groups were compared separately with those from Caucasian patients, the differences in cyclin D1 overexpression did not reach statistical significance ($p=0.235$). This may have been due to the small number of cases in each subset; there were only 19 AA, 24 Hispanic, and 5 Asian cases.

Because our previous study included only a small number of AA cases, we expanded this analysis by establishing a tumor bank containing samples from approximately 100 cases of breast cancer obtained from AA women, and 100 cases obtained from Caucasian women. The women were matched on age, stage at presentation, date of diagnosis, and ER status. Matching was performed to minimize the confounding effects of demographic and clinicopathologic variables on the endpoints of this study. As part of our expanded study, we also examined other critical cell cycle control proteins – p53, $p27^{Kip1}$ (p27), and $p21^{Cip1}$ (p21) – that have been previously studied in breast cancer (15-18). In addition, we have collected clinical outcome and survival data on the majority of cases in this cohort, to evaluate the possible prognostic value of these proteins in both groups of breast cancer cases.

Materials and Methods

Case selection and clinicopathologic variables. Two hundred cases of invasive breast cancer were identified from the New York Presbyterian Hospital (NYPH) Breast Cancer Registry, USA. Clinical specimens were obtained at surgery between 1990 and 1998 and were processed by routine histopathologic methods. Cases from approximately 100 AA and 100 Caucasian women were selected and matched on patient age, disease stage (American Joint Committee on Cancer (AJCC) staging system), ER status, and year of diagnosis (within 5 years). The last criterion was chosen to adjust for variations in the methods of measuring ER expression and other variables that are routinely analyzed for each breast cancer specimen. Self-reported and physician-reported classifications of race were used to identify cases. For each case, formalin-fixed, paraffin-embedded sections were retrieved from the Department of Pathology, and five immunoblanks were prepared. Patient identifiers, including name and hospital medical record number, were not used in order to ensure patient confidentiality. This project was submitted to and approved by the Columbia-Presbyterian Medical Center Institutional Review Board (IRB) prior to specimen acquisition (IRB #X0898). For each histological section, information regarding the following variables was also obtained from the Department of Pathology: tumor grade (grade 1 – well-, grade 2 – moderately-, grade 3 – poorly- differentiated), S-phase fraction (low – less than 6%, intermediate – between 6-10%, high – greater than 10%), proliferation index (MIB-positive – greater than or equal to 20%, -negative – less than 20%), Her-2/neu expression (positive – greater than 0.1 pg/ml, negative – less than 0.1 pg/ml), and progesterone receptor (PR) status (positive or negative). These variables were treated as covariates. The methods used to measure these covariates, which are routinely conducted for each breast cancer case at our institution, have been previously described (19).

Clinical outcome data. The NYPH Breast Cancer Registry actively collects and updates cancer recurrence and survival information on its cases. As permitted under registry guidelines, information is obtained through periodic telephone contacts with patients and through the computerized medical record database at NYPH. Clinical outcome information was supplied by the registry according to a confidential registry accession number that is unique to each case. The registry provided the following outcome information: date of first recurrence, first recurrence type (none, disease-free, distant recurrence, local recurrence, unknown recurrence status), cancer status (no evidence of this cancer, evidence of this cancer, unknown), date of the last patient contact, and survival quality (normal activity, dead, unknown/unspecified). In the analysis, we did not distinguish between local or distant recurrence. For each case, race and tumor grade information were used to crosscheck the Registry and Department of Pathology databases.

Immunohistochemistry (IHC) – protein biomarker expression. The levels of protein expression of cyclin D1, p53, p27, and p21 were the main endpoints of this study. All IHC analyses were performed using an avidin-biotin complex immunoperoxidase technique, as previously described (20, 21). Briefly, five micrometer, formalin-fixed, paraffin-embedded tissue sections were placed on silane-coated slides and baked at 60˚C for 30 minutes, deparaffinized, hydrated, placed in 10 mM citrate buffer (pH 6) and microwaved for a total of 25 minutes (antigen retrieval). Slides were blocked with horse serum, and then incubated with the appropriate primary antibody: cyclin D1 antibody (clone 5D4, 1:200 dilution; Immunotech, Westbrook, ME, USA), p53 mouse monoclonal antibody (clone D01, 1:5 dilution; Immunotech), p27 mouse monoclonal antibody (clone 57, 1:350 dilution; Transduction Laboratories, Lexington, KY, USA), and p21 mouse monoclonal antibody (clone EA10, 1:50 dilution; Oncogene, Cambridge, MA, USA). Immunostaining was performed on a DAKO autostainer, using a Vector biotinylated secondary anti-mouse antibody (dilution 1:200 for 30 minutes) and an avidin-biotin peroxidase complex for detection (Vectastain Elite, Vector Laboratories, Burlingame, CA, USA). The chromogen diaminobenzidine (DAB) was utilized and sections were counterstained with methyl green (ethyl green) (Sigma, St. Louis, MO, USA). Appropriate positive and negative (immunostaining lacking primary antibodies) controls were used in each batch of staining. Slides were counterstained with hematoxylin. All sections were scored for protein expression in a blinded fashion by two pathologists (L.M. and H.H.). The particular terminology ("expression" *versus* "overexpression") and scoring system were specific for each protein and based on previously reported studies.

Nuclear staining of cyclin D1, p53, p27, and p21 in the cases of breast cancer was evaluated by a semi-quantitative scoring system that considers staining intensity and percentage of positive nuclei. The system assesses nuclear staining intensity as a 4-level ordered categorical variable (0=none, 1=mild, 2=moderate, 3=strong), and the percentage of positive cells as a 5-level ordered categorical variable (cyclin D1, p53, and p21: 0=none or rare cells, $1 \leq 10$ %, 2=10-25%, 3=25-50%, $4 \geq 50$%; p27: 0=none or rare cells, $1 \leq 25$ %, 2=25-50%, 3=50-75%, $4 \geq 75$%). The assignment of "positive" and "negative" values was based on previous studies. Cases were considered "positive" for cyclin D1 "overexpression" if the nuclear staining had an intensity score of "moderate" or "strong", and at least 10% or more of tumor cells demonstrated evidence of staining.

Table I. *Results of univariate analyses of demographic and clinicopathologic variables.*

			Race				
			African-American		Caucasian		
			n	%	n	%	*p*
Total cases			98		101		
Age at diagnosis (years)		< 50	23	23	21	21	0.65
		≥ 50	75	77	80	79	
Mean age (range)			62.5	(31-94)	62.4	(33-94)	0.96
Stage of disease		I	38	39	41	41	0.78
		II	56	57	54	53	
		III & IV	4	4	6	6	
Grade		1	9	10	6	6	0.50
		2	40	43	40	40	
		3	44	47	54	54	
Estrogen receptor		positive	67	73	68	69	0.53
		negative	25	27	31	31	
Progesterone receptor		positive	55	60	58	59	0.87
		negative	37	40	41	41	
S-phase fraction	Low	< 6%	27	32	26	29	0.37
	Intermediate	6-10%	24	29	20	22	
	High	> 10%	32	39	44	49	
Proliferation index (MIB-1)		≥ 20	29	34	32	35	0.88
		< 20	57	66	60	65	
Her-2/neu expression		positive	31	32	35	35	0.65
		negative	66	68	65	65	
Disease recurrence		positive	26	33	17	27	0.41
		negative	53	67	47	73	
Survival		alive	53	62	60	68	0.36
		dead	33	38	28	32	
Protein expression							
Cyclin D1		positive	46	49	45	49	0.94
		negative	48	51	46	51	
p53		positive	33	36	35	38	0.80
		negative	59	64	58	62	
p27		positive	41	43	41	44	0.85
		negative	55	57	52	56	
p21		positive	53	55	50	50	0.42
		negative	43	45	51	50	

Cases were considered "positive" for p53 "expression" if the nuclear staining had an intensity score of "moderate" or "strong", and at least 10% or more of tumor cells demonstrated evidence of staining. In most studies, p53 "overexpression" is not distinguished from "expression" (22, 23). High expression of p27 was distinguished from low or loss of expression, which is consistent with previous investigations (24-26). Cases were considered "positive" if the nuclear staining had an intensity score of "strong" and at least 50% of tumor cells demonstrated evidence of expression, or if the staining had an intensity score of "moderate" and at least 75% of cells displayed p27 expression. All other combinations of scoring were considered "negative" for p27 expression. Recently, cytoplasmic levels of p27 were found to correlate with cancer "aggressiveness" in a collection of breast cancer specimens (27). In the current study, only nuclear levels of p27 were measured. Cases were considered "positive" for p21 "expression" when more then "rare" tumor cells displayed at least "mild" intensity staining. As with previously reported studies, we did not further distinguish those cases with "overexpression" of p21 (28-30). The rationale for the various cut-off points also takes into account the "negative" background level of staining of the adjacent normal, non-neoplastic cells present on sections of breast tissue.

Statistical methods. The primary endpoints in this study were the rates of cyclin D1 overexpression and p53, p27, and p21 expression in the two groups of breast cancer cases – AA *versus* Caucasian.

Her-2/neu expression, S-phase fraction, proliferation index (MIB-1), and tumor grade were treated as covariates in the analyses. Univariate analyses were performed to assess the association of cyclin D1, p27, p53, and p21 expression with race and the other clinicopathologic parameters. In general, the Fisher's exact *t*-test was used to assess associations with binary factors and unordered, multi-level factors, and the Mantel-Haenszel Chi-squared test was used to assess associations with ordered, multi-level factors. Specifically, Chi-squared analysis and the Fisher's exact *t*-test were used to compare the study outcomes and covariates by race. Chi-squared analysis was also used to investigate correlations between protein expression and ER status. Associations with factors that were significant at p values less than 0.15 in univariate analysis were entered into a multiple logistic regression analysis. Stepwise forward selection was used to select which variables to include in the final regression models. Cumulative logistic regression was used to evaluate the role of each outcome and covariate in predicting cancer stage. Odds ratios (OR) and 95% confidence intervals (CI) were calculated. Cox proportional hazards regression was used to evaluate the prognostic value of each protein and covariate in predicting overall survival and disease-free survival (cancer recurrence). Overall and disease-specific survival times were calculated from the date of diagnosis to the date of death and the earlier of cancer recurrence and death, respectively. For each analysis, the event hazard ratio (HR) and 95% CI were determined. Overall and disease-free survival curves were also computed using the Kaplan-Meier method; the log-rank test was used to determine significance ($p<0.05$). All analyses were performed using the SAS System (Version 6.09, SAS Institute, Cary, NC, USA).

Results

Patient characteristics and protein expression rates. Among the 199 cases of female breast cancer, there were 98 AA and 101 Caucasian cases (Table I). In the majority of cases, the following parameters were known: race, age, stage (AJCC stages I-IV), date of diagnosis, grade, ER status, PR status, S-phase fraction, MIB-1, and level of Her-2/neu expression. Clinical and pathologic information were available for most, but not all, specimens. Therefore, the total number of specimens was not always 199 for each category. Univariate analyses were performed to determine if there were any differences in the various clinical and pathologic variables between the AA and Caucasian cases. The mean age of each population (AA and Caucasian) was 62 years. The tumor bank was well-matched on age, stage, date of diagnosis, and estrogen receptor status (all p values >0.5). The majority of cases in both groups were early-stage (Stage I or II) (96% AA, 94% Caucasian), ER-positive (73% AA, 69% Caucasian), PR-positive (60% AA, 59% Caucasian), and Her-2/neu-negative (68% AA, 65% Caucasian). There were no significant differences in tumor grade, S-phase fraction, MIB-1, Her-2/neu expression, and PR status between the two populations ($p>0.3$; Table I).

Cyclin D1, p53, p27, and p21 expression rates, the primary study endpoints, were similar in matched cases of AA and Caucasian breast cancer ($p>0.4$). Thus, this larger analysis does not confirm our previous finding of increased cyclin D1 overexpression in non-Caucasian breast cancer (14). Furthermore, because the rates of cyclin D1 positivity and negativity were similar (approximately 50%; Table I), it is not likely that increasing the sample size further would reveal any differences in the frequency of cyclin D1 overexpression. Follow-up information on survival and disease recurrence were obtained for 87% and 71% of the cases, respectively. After a median follow-up of 7 years, 62% of AAs and 68% of Caucasians were alive, while 33% of AAs and 27% of Caucasians had evidence of cancer recurrence. Thus, in our series, although the clinical outcome appeared to be slightly worse among AAs, there were no significant racial differences in cancer recurrence or survival ($p>0.3$; Table I). Since age, stage, ER status, and diagnosis date were used to match these cases, univariate analyses of these variables were not performed.

Cyclin D1 overexpression was significantly associated with ER expression in Caucasian but not AA cases. Univariate analyses were performed to investigate the associations between cell cycle protein expression and ER positivity in the cases of breast cancer (Table II). Previous reports have demonstrated that cyclin D1 overexpression is associated with ER expression (31), and that p53 expression is inversely associated with ER status (22, 23, 32). In the Caucasian samples, cyclin D1 overexpression was strongly associated with ER expression (OR=5.43; $p=0.0005$). In the AA cases, however, cyclin D1 expression was not significantly associated with ER expression (OR=2.41; $p=0.07$). Expression of the p53 protein was inversely associated with ER expression in both Caucasian (OR=0.13; $p<0.0001$) and AA (OR=0.38; $p=0.04$) cases; however, this association was much stronger in the Caucasian samples. These are expected findings, since the majority of previous IHC-based reports that demonstrated associations between cyclin D1, p53, and ER status were presumably comprised of samples from mainly Caucasian patients. The expression of p27 was strongly associated with ER expression in both AA ($p=0.008$) and Caucasian ($p=0.0004$) cases, and p21 expression was not associated with ER expression in either group. These results are consistent with previously reported studies (25, 29, 33). Thus, although cyclin D1 overexpression was similar in the two populations, it was significantly associated with ER expression in only the Caucasian samples and not in the AA samples. Although the clinical relevance of this finding is presently unclear, it represents a potentially new difference in tumor biology that may distinguish the breast cancers that develop in AA women from those that develop in Caucasians.

Table II. *Associations between protein expression and estrogen receptor status.*

	Estrogen Receptor Status						
	positive		negative		OR	95% CI	*p*
	n	%	n	%			
African-American							
cyclin D1					2.41	0.90–6.44	0.07
Positive	35	55	8	33			
Negative	29	45	16	67			
p53					0.38	0.14–0.98	0.04
Positive	18	29	13	52			
Negative	44	71	12	48			
p27					4.17	1.39–12.5	0.008
Positive	34	52	5	21			
Negative	31	48	19	79			
p21					1.89	0.73–4.87	0.18
Positive	40	62	11	46			
Negative	25	38	13	54			
Caucasian							
cyclin D1					5.43	2.01–14.6	0.0005
Positive	38	62	7	23			
Negative	23	38	23	77			
p53					0.13	0.05–0.34	<.0001
Positive	14	22	20	69			
Negative	49	78	9	31			
p27					6.22	2.10–18.4	0.0004
Positive	35	56	5	17			
Negative	27	44	24	83			
p21					1.29	0.55– 3.02	0.56
Positive	35	51	14	45			
Negative	33	49	17	55			

Subset analyses. We conducted additional analyses to investigate the decreased association between cyclin D1 overexpression and ER positivity that occurred in the AA cases. Estrogen has been postulated to induce cyclin D1 expression through a specific response element in the cyclin D1 promoter (34). In the absence of the ER, Her-2/neu can also stimulate cyclin D1 expression through mitogen-activated protein kinase and AP-1 signal transduction pathways (35, 36). Thus, we investigated the level of Her-2/neu expression in the subset of ER-negative cases in both AA and Caucasian groups (data not shown). This is a particularly relevant group of patients, since AA breast cancer patients more often develop cancers that are ER-negative (3). There was no significant difference in Her-2/neu expression in the AA and Caucasian ER-negative cases, although there were only 25 AA and 31 Caucasian cases in this ER-negative subset (data not shown). Similarly, there were no differences in the expression levels of the four cell cycle proteins and in the PR status, S-phase fraction, MIB-1, disease recurrence, or survival values between the ER-negative AA and Caucasian cases (data not shown). As expected, the ER-negative cases were more often cyclin D1-negative (67% AA, 77% Caucasian), p53-positive (52% AA, 69% Caucasian), and p27-negative (79% AA, 83% Caucasian) (data not shown).

Because the proportion of younger-aged women who develop breast cancer is higher among AAs (2), we conducted similar analyses in the subset of cases obtained from "younger" women (age ≤ 50 years). In this subset, the proportion of ER-positive tumors was much lower than that

in the original dataset (57% AA and Caucasian (data not shown)) compared with approximately 70% (Table I). These results are consistent with previous evidence that breast cancer that develops in younger women is more often ER-negative (26). There was also a corresponding decrease in the proportion of cyclin D1- and p27-positive tumors (data not shown). There were no significant differences in ER and PR status; S-phase fraction, MIB-1, and Her-2/neu expression; disease recurrence and survival; and the expression levels of cyclin D1, p53, p27, and p21 between AA and Caucasian cases. Cyclin D1 overexpression and p53 expression were strongly associated with ER status in the Caucasian cases in this analysis ($p<0.05$). Expression of neither cyclin D1 nor p53 was associated with ER status in the AA subset.

Multivariate regression analyses were performed to investigate the value of each protein (cyclin D1, p53, p27, p21) and each covariate (tumor grade, S-phase fraction, MIB-1, PR status, Her-2/neu expression) in predicting the following clinical endpoints: disease stage at diagnosis, disease-free survival, and overall survival. When the AA cases were compared with the Caucasian cases, there were no clear patterns regarding the predictive value of any of the cell cycle related proteins (data not shown). Therefore, we were unable to determine the prognostic role of these proteins in either the AA or Caucasian cases. Cancer recurrence information, which was used to calculate disease-free survival, was available for only 81% of the AA and 63% of the Caucasian cases (Table I). In addition, only 4% of the AA and 6% of the Caucasian cases were either stage III or IV, thus limiting the power of the stage analysis.

Discussion

We have created a matched tumor bank containing approximately 200 cases of invasive breast cancer. These cases were obtained from approximately equal numbers of AA and Caucasian patients, and were matched on age at diagnosis, stage at diagnosis, ER status, and date of diagnosis. In our IHC-based analyses of four cell cycle control proteins, we found that cyclin D1, p53, p27, and p21 expression rates were similar in matched cases of AA and Caucasian breast cancer and similar to values reported in previous studies (22-26, 28-30, 32, 37-41). However, we found that although cyclin D1 overexpression was associated with ER status in the Caucasian cases, this did not appear to be true in the AA cases. The clinical relevance of this novel finding is not clear at the present time, but it may represent a true difference in tumor biology in AA breast cancer.

Racial and ethnic differences in breast cancer prognosis and survival have been studied extensively. The majority of these studies have investigated differences in demographic characteristics, clinical factors, and tumor pathology between Caucasians and AAs (reviewed in (3, 42)). AAs with breast cancer have consistently shown a shortened survival, but it is not clear whether this is due to socioeconomic differences or to racial differences in tumor biology. The interactions between race and ethnicity, socioeconomic differences, disease stage at time of diagnosis, and actual differences in tumor biology are complex, and the various current hypotheses and findings, which are based on retrospective data, are not definitive. What is known is that breast cancer that develops in AAs, when compared to Caucasians, is more frequently of higher tumor grade (43, 44), more frequently of the medullary subtype (2), and less frequently of the lobular subtype (45). In addition, numerous reports have demonstrated that breast cancers that develop in AA patients less frequently express the ER (3, 43, 44, 46). This finding is of clinical importance, since it suggests that AA breast cancer may be less responsive to hormonal manipulation. However, these differences have not thus far been helpful in designing specific target-based management or prevention strategies.

In a previous study, we obtained data that suggested that cyclin D1 overexpression was more prevalent in non-Caucasian cases of breast cancer (14). However, this finding was only of borderline significance ($p=0.051$), mainly due to the small number of non-Caucasian cases in this study. Therefore, we conducted the current more extensive comparative study of AA and Caucasian breast cancer to confirm whether cyclin D1 overexpression is indeed significantly more frequent in AA breast cancer. In this study, cyclin D1 overexpression was present in 49% of both the AA and Caucasian cases (Table I). This value is within the range reported in previous studies that were probably composed of predominantly Caucasian cases (31). Therefore, the current larger and higher powered study does not confirm our previous finding, suggesting that cyclin D1 overexpression is more frequent in AA cases of breast cancer.

As discussed above, ER-positive breast cancers are usually cyclin D1-positive and p53-negative (23, 31). The samples used in these previously reported series were presumably obtained from predominantly Caucasian cases, although race and ethnicity data were often not provided. This may explain our finding in the present study that ER status did not have a significant association with cyclin D1 overexpression in our series of AA breast cancer. The mechanistic and clinical implications of this lack of association are not clear. Experimental studies indicate that estrogen can induce cyclin D1 (34) and, conversely, cyclin D1 can stimulate ER transcriptional activity (47). Perhaps one or both of these mechanisms are less likely to occur in AA breast cancer. A potential explanation could be the earlier onset of menarche in AA adolescent girls (48). This earlier exposure of breast tissue to the unopposed action of estrogens might somehow promote the development of ER-independent, cyclin D1-

dependent breast cell proliferation and breast carcinogenesis. This mechanism may also explain the predisposition of AA women for developing ER-negative breast cancers at an early age (2, 4). If in AA women cyclin D1 overexpression represents an early estrogen-independent event during breast carcinogenesis and contributes to the more frequent development of aggressive, higher grade cancers, inhibiting its overproduction or activity might provide a target-specific approach to the prevention and treatment of breast cancer in this population.

Porter and colleagues recently presented a very similar and important study in which they investigated the expression of cell cycle-related proteins in cases of breast cancer obtained from AA and Caucasian patients (49). Among their findings, the authors reported the following among the AA cases: higher grade, loss of ER expression, overexpression of p53, and less frequent cyclin D1 expression. Because the cases in their study were not matched, the proportion of ER-negative cancers was expectedly much higher among the AA cases (62.8% compared with 37.4% Caucasian cases) (3, 49). Thus, their findings of p53 overexpression and less frequent cyclin D1 expression among the AA cases may be explained in part by the higher prevalence of ER-negative tumors based on previously described associations between these three proteins (22, 23, 31, 32). Because our cases were matched, the proportion of higher grade, ER-negative, cyclin D1-negative, and p53-positive cancers was similar in both the AA and Caucasian cases.

We should, however, emphasize certain limitations of the present study. The first is that most of the specimens in our bank were obtained from women in their seventh decade of life (mean age of 62 years) (Table I). Consequently, the majority of cases are ER-positive, PR-positive, and Her-2/neu-negative, as would be expected in this older population (50). These favorable prognostic features could explain the early-stage presentation of these cases. Furthermore, the majority of patients were alive (62% AA, 68% Caucasian) and free of cancer recurrence (67% AA, 73% Caucasian) at their last follow-up evaluation. Thus, the characteristics of this study population may not fully reflect the younger, ER-negative cases of breast cancer that are reported to be of higher frequency in the AA population (for review, see (2)). In our subset analyses of younger (age $<$ 50 years) and ER-negative cases, we did not discover any unique findings, although these analyses were expectedly underpowered. The large majority of cases in our series were either stage I or II cancers, and there were very few stage III or IV cases. In addition, recurrence and survival data were missing in 28% and 13% of the cases, respectively. Thus, we were not able to draw any significant conclusions regarding the prognostic value of any of the cell cycle proteins in either the AA or the Caucasian cases.

Finally, we should emphasize that, in this study, our classification of cases of breast cancer into two racial categories, AA and Caucasian, was based simply on patient self-identification. There is an ongoing debate as to whether race is an appropriate and valid research variable in biological studies (for review, refer to (51)). Race has been used as a proxy for both genetic similarities and non-genetic factors including socioeconomic status and environmental exposures. Racial and ethnic classifications often imply genetic homogeneity in the specific population being defined (52). Failing to recognize the inherent heterogeneity of populations can compromise the statistical power and conclusions of such studies. Furthermore, racial groupings are often used inconsistently in the literature, thus leading to confusion in both the reporting and interpretation of data. In any case, previous investigators have been able to demonstrate significant racial differences in disease states using existing, although imperfect, categories.

In view of the above considerations, at the present time, it is not clear whether our finding that cyclin D1 overexpression is significantly associated with ER expression in Caucasian but not in AA cases of breast cancer truly reflects inherited genetic differences between these two groups of patients or acquired differences related to socioeconomic status or environmental factors. Nevertheless, if these differences are confirmed they may, as suggested above, propose specific approaches for the prevention and/or treatment of breast cancer in AA women. The findings of our and Porter's studies may help to determine the role of tumor biology in the generally worse prognosis of AA breast cancer patients.

Acknowledgements

We wish to acknowledge Dr. I. Bernard Weinstein for invaluable advice during the preparation of this manuscript. We also thank Miguel Guerrero, Judith Oniel, and Aaron Trimpin from the Breast Cancer Registry; Rongzhen Chen and Yang Lin from the Department of Pathology; and Barbara Castro and Javier Delgado from the Herbert Irving Comprehensive Cancer Center for assistance with this project. This research was supported by awards from the Susan G. Komen Breast Cancer Foundation, the Jean Sindab African American Breast Cancer Project, and the Avon Foundation.

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Received September 9, 2004
Accepted December 10, 2004