Household Income Is Associated with the p53 Mutation Frequency in Human Breast Tumors

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Abstract

Background: A study from Scotland reported that the p53 mutation frequency in breast tumors is associated with socio-economic deprivation.

Methods: We analyzed the association of the tumor p53 mutational status with tumor characteristics, education, and self-reported annual household income (HI) among 173 breast cancer patients from the greater Baltimore area, United States.

Results: p53 mutational frequency was significantly associated with HI. Patients with < $15,000 HI had the highest p53 mutation frequency (21%), followed by the income group between $15,000 and $60,000 (18%), while those above $60,000 HI had the fewest mutations (5%). When dichotomized at $60,000, 26 out of 135 patients in the low income category had acquired a p53 mutation, while only 2 out of 38 with a high income carried a mutation ($P < 0.05$). In the adjusted logistic regression analysis with 3 income categories (trend test), the association between HI and p53 mutational status was independent of tumor characteristics, age, race/ethnicity, tobacco smoking and body mass. Further analyses revealed that HI may impact the p53 mutational frequency preferentially in patients who develop an estrogen receptor (ER)-negative disease. Within this group, 42% of the low income patients (< $15,000 HI) carried a mutation, followed by the middle income group (21%), while those above $60,000 HI did not carry mutations ($P_{trend} < 0.05$).

Conclusions: HI is associated with the p53 mutational frequency in patients who develop an ER-negative disease. Furthermore, high income patients may acquire fewer p53 mutations than other patients, suggesting that lifetime exposures associated with socio-economic status may impact breast cancer biology.

Introduction

Breast cancer incidence and mortality rates show large differences among population groups within the United States (US) and between more and less developed countries worldwide [1–3]. Results from cancer epidemiology indicate that differences in reproductive history and exposures to certain lifestyle factors largely explain geographic and race/ethnic variations in the breast cancer incidence [4]. In contrast, population differences in mortality are thought to be caused by inadequate and delayed access to health care and by differences in disease presentation at diagnosis [5–10]. Recently, it has been argued that additional, yet unrecognized differences in tumor biology may exist that account for some of the observed race/ethnic differences in disease survival in the US [11,12].

It has been well established that a patient’s socioeconomic status (SES) is associated with breast cancer survival and may influence tumor characteristics like the mutational and estrogen receptor (ER) status of tumors. For example, women from a low SES background were found to be more likely to develop an ER-negative disease than those from a high SES background [13,14]. Other studies described race/ethnic differences in the p53 tumor suppressor status of breast cancer patients in the US [15,16], suggesting environmental and SES-related influences on tumor biology that lead to these differences. This hypothesis is consistent with the literature showing that the tumor p53 mutational status has an environmental signature and can sometimes be traced back to well defined exposures [17].

A study of 246 breast cancer patients in Scotland recently reported that the p53 mutational frequency in breast tumors is associated with socio-economic deprivation [18]. Women with the highest deprivation scores in the area of their residence were found to acquire p53 mutations more frequently than those living in other areas. This finding links socio-economic deprivation to a poor outcome phenotype because the presence of a p53 mutation in breast tumors confers decreased disease-free and overall survival [19,20]. While intriguing, observations like these will need further
validation in other patient groups. Thus, we tested the hypothesis that annual household income (HI), as a measure of SES, is associated with the p53 mutation frequency in a cohort of US breast cancer patients. Our study found that HI was associated with the p53 mutation frequency in breast tumors, and high income patients may acquire fewer p53 mutations than other patients. Moreover, our data revealed that HI may impact the p53 mutational frequency preferentially in patients who develop an ER-negative disease.

**Results**

We previously established a well characterized cohort of 143 African-American and 105 European-American breast cancer patients with information on the tumor p53 mutational status and survival follow-up [21]. This patient population was recruited in the greater Baltimore area in Maryland, US, and is representative of an inner city, low income community with a large minority population. Many of the patients were from an impoverished background and 47 (27%) of them reported annual incomes less than $15,000. The high proportion of African-American patients explains the elevated frequency of high grade and ER-negative tumors in this patient population, which is representative of African-American breast cancer patients [4,7,22]. Self-reported HI was available for 173 of the 248 patients (70%). Comparing the patients with HI information (n = 173) versus those without this information (n = 75), there was no difference with respect to self-reported race/ethnicity (P = 0.78), tumor ER status (P = 1.0), node status (P = 0.77), or body mass index (P = 0.74), but patients with missing income information tended to be older (57.7 versus 54.0 mean age; P = 0.05) and their tumors tended to have a higher grade (58% versus 46%; P = 0.14) and a higher p53 mutation frequency (26.7% versus 16.2%; P = 0.08).

To evaluate the association between tumor p53 status and selected patient and tumor characteristics, patients were stratified into no/yes mutation carriers and the association with tumor and patient characteristics was assessed (Table 1). The tumor p53 status was significantly associated with HI, node status, tumor ER status, number of tumor-infiltrating macrophages, and reached the P = 0.05 significance level with tumor grade. Tobacco consumption in pack years tended to be higher in patients without p53 mutations while African-American patients were more likely to acquire a p53 mutation (20.4%) than European-American patients (10.7%), albeit this relationship was not statistically significant (P = 0.1). An increased p53 mutation frequency in ER-negative and high grade tumors has been observed by others [23,24], consistent with our findings. Somewhat unexpected was the inverse relationship between p53 mutations and disease node status in our study cohort, whereas other studies either did not find an association between the two variables [23,24] or observed a positive relationship [18]. There was no association between p53 mutation frequency and age at diagnosis, education, or a patient’s body mass index (BMI). Patients with less than $15,000 HI had the highest p53 mutation frequency (10/47; 21%), followed by the income group between $15,000 and $60,000 (16/68; 18%), while those above $60,000 HI had the fewest mutations (2/38; 5%) [trend test: P = 0.057 using logistic regression with income coded 0,1,2]. In the multivariable logistic regression analysis with 3 income categories, HI was significantly associated with the p53 mutation frequency after adjustment for node status, tumor ER status, tumor grade, and race/ethnicity [odds ratio (OR) = 0.42, 95% CI: 0.18 to 0.97 for acquiring a tumor p53 mutation with increasing HI]. This association remained significant when tobacco consumption and additional demographic variables (age, education, BMI) were added to the model (Table 2). In contrast to the tumor p53 mutational status, we did not observe an independent association between HI and either tumor p53 protein expression or the tumor ER status. Aberrant accumulation of nuclear p53 protein is commonly associated with the presence of a p53 mutation although the prognostic significance of nuclear p53 expression in breast tumors has been questioned [19,20]. HI and aberrant nuclear p53 accumulation in the breast tumors were inversely related in this study, but in contrast to the relationship between HI and the tumor p53 mutational status, this association was not significant in the multivariable analysis (Table 2). Finally, we explored whether the tumor p53 mutational status is associated with race/ethnicity in the adjusted logistic regression analysis because African-American patients tended to acquire a p53 mutation more commonly than European-American patients (Table 1). In the adjusted models, as shown in Table 2, no relationship between race/ethnicity and the tumor p53 mutational status remained (OR = 1.03, 95% CI: 0.43 to 2.45, with adjustments for household income, tumor grade, and ER and node status; OR = 1.05, 95% CI: 0.43 to 2.56, with an additional adjustment for smoking; OR = 1.11, 95% CI: 0.34 to 3.58, with further adjustments for age, education, and BMI). Thus, race/ethnicity was not independently associated with the tumor p53 mutation frequency, whereas household income was.

Because p53 mutations occur most commonly in the ER-negative disease and women from a low SES background were found to be more likely to develop an ER-negative disease than those from a high SES background, we performed an additional analysis of the association between HI and the p53 mutation frequency after stratification of the patients by tumor ER status. This analysis revealed that HI may impact the p53 mutation frequency preferentially in patients who develop the ER-negative disease, but not in ER-positive tumors (Table 3). Within this group of ER-negative patients, 42% of the lowest income patients carried a mutation, followed by the middle income group (21%), while those in the high income group did not carry any mutations (P = 0.044).

In an exploratory approach, we also examined the association of HI and the tumor p53 status with disease-specific survival using Cox regression modeling. Both HI [hazard ratio (HR) = 0.64, 95% CI: 0.44 to 0.94 for dying from breast cancer with increasing HI] and the tumor p53 mutation status (HR = 1.66, 95% CI: 1.02 to 2.7 for a p53 mutation carrier vs. non-carrier) were significantly associated with survival in the univariable analysis, but not education (HR = 0.76, 95% CI: 0.45 to 1.26). Figure 1 shows a Kaplan-Meier plot of the relationship between HI and breast cancer-specific survival. In an analysis that included both HI and tumor p53 mutation status as covariates, only HI was a significant predictor of survival. The inclusion of other covariates to the model that were associated with survival in the univariable analysis (age, TNM stage, ER status in addition to HI and p53 mutation status) yielded a borderline significant association for both HI (HR = 0.62, 95% CI: 0.38 to 1.02 for dying from breast cancer with increasing HI) and tumor p53 status (HR = 1.95, 95% CI: 0.93 to 4.1 for a p53 mutation carrier vs. non-carrier) with disease-specific survival, suggesting that these two variables are likely independent predictors of survival in larger studies. Additional analyses did not find that the two variables may affect survival through an interaction.

**Discussion**

A mutation in the p53 tumor suppressor gene has been linked to poor disease outcome and therapy resistance in breast cancer...
Thus, exposures that promote the development of p53 mutations will negatively affect therapy response and disease survival of breast cancer patients. Other studies observed that the p53 mutational spectrum in various human cancers can be linked to distinct carcinogen exposures like aflatoxin exposure and tobacco consumption, and unique mutational spectra have been observed in certain geographic area [17,27], showing that the environment can influence tumor biology by affecting the tumor p53 mutational status.

Here, we report the finding that HI as a measure for individual-level SES is inversely associated with the p53 mutational frequency in breast cancer patients from the greater Baltimore area in the US, which corroborates previous observations from a study in Scotland showing that community-level SES had a similar effect [18]. Thus, socioeconomic factors may affect breast cancer biology by either increasing the risk of acquiring a tumor p53 mutation among those with a low income or decreasing it for those with a high income. We also discovered that HI was rather distinctively associated with the p53 mutational frequency in patients who develop an ER-negative disease, but not in those with the ER-positive disease (mostly luminal A & B tumors). This novel observation may at least be partly explained by the close relationship between the occurrence of a p53 mutation and the development of basal-like and triple-negative breast tumors [28,29]. Thus, exposures associated with SES may specifically impact the breast tumor biology and development of ER-negative breast cancer subtypes by selecting for mutant p53 tumors, consistent with several epidemiologic studies that linked a low SES to an increased risk of developing ER-negative tumors [13,14].

Our observation that high income patients acquired significantly fewer p53 mutations in their primary tumors than other patients contrasts with the findings in the Scottish study where

| Table 1. Association of patient characteristics with tumor p53 mutational status. |
|---------------------------------------------------------------|
| All Cases (n = 173) | p53 mutation No (n = 145) | p53 mutation Yes (n = 28) | P value* t-test |
|--------------------------|--------------------------|--------------------------|----------------|
| **Age at diagnosis (mean ± SD; n = 173)** | 54.0 ± 13.3 | 54.0 ± 13.2 | 53.6 ± 14.4 | 0.89 |
| **Body mass index (mean ± SD; n = 168)** | 28.9 ± 8.4 | 28.9 ± 8.7 | 29.1 ± 6.4 | 0.93 |
| **Tumor-associated macrophages (CD68)** | 97.5 ± 58.6 | 92.9 ± 57.3 | 120.8 ± 60.6 | 0.021 |
| **Smoking in pack years (mean ± range; n = 164)** | 9.8 (0–112) | 10.3 (0–112) | 7.6 (0–111) | 0.08*** |
| Race/ethnicity | | | | |
| African-American | 98 | 78 (80) | 20 (20) | 0.10 |
| European-American | 75 | 67 (89) | 8 (11) | 0.045 |
| **Household income** | | | | |
| ≤ $60k/year | 135 | 109 (81) | 26 (19) | 1.0 |
| > $60k/year | 38 | 36 (95) | 2 (5) | 0.009 |
| **Education** | | | | |
| No High school degree | 39 | 33 (85) | 6 (15) | 0.034 |
| High school degree or more | 132 | 110 (83) | 22 (17) | 0.073 |
| **Stage at diagnosis (TNM)** | | | | |
| ≤ Stage II | 131 | 108 (82) | 23 (18) | 1.0 |
| Stage III/IV | 25 | 21 (84) | 4 (16) | 0.011 |
| **Node status** | | | | |
| Negative | 99 | 77 (78) | 22 (22) | <0.001 |
| Positive | 61 | 57 (93) | 4 (7) | <0.001 |
| **Tumor grade** | | | | |
| Low (1 & 2) | 81 | 72 (89) | 9 (11) | 0.050 |
| High (3) | 70 | 53 (76) | 17 (24) | 0.034 |
| **Estrogen receptor** | | | | |
| Negative | 71 | 54 (76) | 17 (24) | 0.034 |
| Positive | 102 | 91 (89) | 11 (11) | 0.001 |
| **p53 IHC** | | | | |
| Negative | 119 | 108 (91) | 11 (9) | 0.001 |
| Positive | 54 | 37 (69) | 17 (31) | 0.08*** |

*P value comparing patient characteristics by tumor p53 mutational status.
*Cases with missing information are not included.
**Mann-Whitney rank sum test.
Ann Arbor in-house income, race/ethnicity, and education are self-reported. Tumor-associated macrophages were counted as CD68-positive cells. Pack years: (packs smoked per day) x (years as a smoker). BMI = kg/m²; SD = standard deviation, IHC = immunohistochemistry.

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Currently, we do not know the mechanism by which socioeconomic deprivation may increase the risk of acquiring a p53 mutation in breast tissue. It is possible that certain environmental exposures associated with socio-economic deprivation impact breast tumor biology. For example, hormone replacement therapy, lack of physical activity, and increased alcohol consumption are recognized breast cancer risk factors [30,31]. Exposure to these risk factors may vary between population groups [32]. An association between alcohol consumption and p53 mutation frequency in breast tumors of premenopausal women has been observed in one study [33] but the mechanism by which alcohol may cause p53 mutations in unknown. Some of these risk factors could indirectly affect the p53 mutation frequency by altering sex hormone metabolism and by increasing estradiol metabolite-induced mutagenesis [34]. One recent publication revealed a relationship between low early-life social class and increased pro-inflammatory signaling in healthy volunteers [35], which is particularly interesting since inflammation is a cancer risk factor and the expression of inflammation-induced enzymes like inducible nitric oxide synthase and activation-induced cytidine deaminase has been associated with an increased p53 mutational frequency [36–39]. Other studies have linked environmental stress exposures to increased disease aggressiveness in breast cancer [40–42]. Psychological stressors that are more common in deprived communities have been shown to have long-lasting effects on immunity and inflammation pathways [43,44]; they may interact with environmental pollutants to increase the rate of p53 mutations in affected individuals and breast cancer patients. Nonetheless, future research is needed to

### Table 2. Relationship between tumor p53 status and annual household income in the adjusted analysis.

| Household Income | Odds of acquiring a p53 mutation with increasing household income* | Odds of acquiring a p53 IHC-positive tumor with increasing household income* |
|------------------|---------------------------------------------------------------|--------------------------------------------------------------------------------|
| Low income       | 0.42 (0.18 to 0.97)                                           | 0.57 (0.30 to 1.08)                                                           |
| $15K to $60K     | 0.63 (0.33 to 1.21)                                           | 0.63 (0.33 to 1.21)                                                           |
| High income      | 0.88 (0.40 to 1.97)                                           | 0.88 (0.40 to 1.97)                                                           |

*OR = odds ratio; CI = confidence interval; IHC = immunohistochemistry.
*Trend test. Shown is the OR for the stepwise increase in household income (reference: low income). Income coded as 0 (< $15,000), 1 ($15,000 to $60,000), and 2 (> $60,000); adjustments: smoking (pack years), age, and body mass index (BMI) were used as continuous data; other covariates were dichotomized for the analysis, as shown in Table 1.
**adjusted for race/ethnicity, node status, tumor estrogen receptor status, and tumor grade.

### Table 3. Association of household income with a mutant p53 tumor status by estrogen receptor status.

| Household Income | All tumors | ER-positive tumors | ER-negative tumors |
|------------------|------------|--------------------|--------------------|
|                  | Wt p53 | Mutant p53 | Wt p53 | Mutant p53 | Wt p53 | Mutant p53 |
| Low income < $15K | 37 (79%) | 10 (21%) | 26 (93%) | 2 (7%) | 11 (58%) | 8 (42%) |
| Middle income $15 to $60K | 72 (82%) | 16 (18%) | 38 (84%) | 7 (16%) | 34 (79%) | 9 (21%) |
| High income > $60K | 36 (95%) | 2 (5%) | 27 (93%) | 2 (7%) | 9 (100%) | 0 (0%) |
| P trend (Fisher’s exact test) | 0.09 | 0.55 | 0.044 |

**adjusted for race/ethnicity, node status, tumor estrogen receptor status, and tumor grade.

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Our study design has strengths and limitations that should be discussed. In contrast to the Scottish study, which used area deprivation scores as a surrogate for a patient's SES, we had access to self-reported income and education data which more accurately represent SES of a patient. One limitation in our study is the relatively small sample size of the patient population and the fact that a subset of the patient population did not provide HI data. Because only 28 patients in the study presented with a p53 mutation, some of the multivariable logistic regression estimates could be unreliable. However, it should be noted that the estimates of the relationship between HI (income coded 0, 1, 2) and the tumor p53 mutational status were relatively stable using logistic regression models starting with the unadjusted analysis (OR = 0.55, 95% CI: 0.3 to 1.02) and then applying the models shown in 2. Moreover, the observed association between HI and the p53 mutation frequency remained statistically significant in the multivariable analysis and our observations are consistent with a previously published report. A second limitation relates to the patient cohort. This cohort is representative of an inner city, low income community with a large minority population. These patients developed ER-negative and high grade disease more commonly than expected for women in the US in general and may not be representative for US and European patient populations. However, the association between HI and the tumor p53 mutation frequency remained statistically significant in the multivariable analysis after adjustments for tumor ER status and grade. Furthermore, one may see it as a strength to find that SES was associated with the tumor p53 status in two different patient populations, one from Baltimore with a large African-American population and one from Scotland with a mostly white population. Thirdly, we did not have adequate exposure data for some breast cancer risk factors like alcohol consumption and hormone replacement therapy and could not examine their relationship with the tumor p53 status in this patient population. Lastly, it is possible that our analysis missed TP53 mutations, although we used three methods (single-stranded conformation polymorphism, the GeneChip p53 assay, and direct sequencing) to detect TP53 mutations in microdissected tumor samples.

In conclusion, our study corroborates a previous observation from a study in Scotland that p53 mutant tumors are more common in breast cancer patients from low income, socially deprived communities than in patients from high SES communities, indicating that lifetime exposures associated with a woman's SES may impact breast tumor biology. The current study extends the previous observations by showing that SES may preferentially impact the biology of the ER-negative disease. These findings are significant in light of the ongoing discussions why women from disadvantaged communities tend to have poorer survival than women from high income area. In addition to the existing disparities in access to care, SES may impact tumor biology, leading to a poor outcome phenotype with mutant p53 that more commonly affects low income patients than high income patients.

Materials and Methods

Patient recruitment and survey data

Unselected breast cancer patients at all disease stages were recruited between February 15, 1993, and August 27, 2003, into a biomarker study under a NCI contract (Resource Collection and Evaluation of Human Tissues and Cells from Donors with an Epidemiology Profile), as described previously [21]. They were recruited at the University of Maryland Medical Center, the Baltimore Veterans Affairs Medical Center, Union Memorial Hospital, Mercy Medical Center, and the Sinai Hospital in Baltimore. They were identified through surgery lists and enrolled into the study prior to surgery. Resected tumor tissue (for p53 mutational analysis) was obtained from 248 patients who had pathologically confirmed breast cancer, were of African-American or European-American descent by self-report, and had been diagnosed with breast cancer within the last 6 months before recruitment, and had, by self-report, no previous history of breast cancer. Patients completed an interviewer-administered questionnaire that evaluated socio-economic variables as part of a larger survey. Self-reported HI was available for 173 (70%) of them, and information on education for 171 (69%). Eighteen patients, or their doctor, refused that the questionnaire is administered and 24 patients could not be interviewed while others refused to complete the income section or were not able to report household income. Combined annual household income before taxes and deductions was collected as either unknown, under $15,000, between $15,000 and $60,000, and above $60,000. Education information was collected as highest grade or level of schooling and how many years of school were completed. Self-reported race/ethnicity was collected as Black (not of Hispanic origin) for African-Americans and White (not of Hispanic origin) for European-Americans. Clinical and pathological information including tumor hormone receptor expression was obtained from medical records and pathology reports and HER2 expression was evaluated by immunohistochemistry as described by us previously [45]. Disease staging was performed according to the tumor–node–metastasis (TNM) classification system of the American Joint Committee on Cancer/ the Union Internationale Contre le Cancer (AJCC/ UICC). The Nottingham system was used to determine tumor grade.

Ethics Statement

All patients signed a consent form. The collection of tumor specimens, survey data, and clinical and pathological information was approved by the University of Maryland Institutional Review Board for the participating institutions (UMD protocol #0290229). IRB approval of this protocol was then obtained at all participating institutions. The research was also reviewed and approved by the NIH Office of Human Subjects Research (OHSR #2249).
**TP53 mutational analysis and immunohistochemistry**

Tumors were screened by single-stranded conformation polymorphism analysis for the presence of somatic p53 mutations, as previously described [46,47]. p53 exons 2–11 were amplified by polymerase chain reaction (PCR) from microdissected, paraffin-embedded tumor tissue, as described [48]. PCR products were denatured into single-stranded DNA and loaded on a Gene Gel Excel gel (Amersham Pharmacia Biotech, Piscataway, NJ). The single-stranded DNA was separated by electrophoresis by use of the GenePhor System (Amersham Pharmacia Biotech, Piscataway, NJ) and visualized by DNA silver staining. When an aberrant DNA band pattern was detected, the PCR product was sequenced to determine whether a mutation was present. Most tumors were also screened for mutations in p53 exons 2–11 with the GeneChip p53 assay (Affymetrix, Santa Clara, CA). This protocol has been previously validated [33,49]. Predicted mutations were scored as described [50]. Nuclear p53 expression was determined immunohistochemically with a 1:100 diluted monoclonal DO-7 antibody (DakoCytomation, Carpinteria, CA; recognizes the N-terminus of the p53 protein); p53 expression was scored positive if >10% of the tumor cells expressed nuclear p53.

**Statistical analysis**

Data analysis was performed using Stata/SE 9.0 (Stata Corp, College Station, TX) statistical software package. All statistical tests were two-sided, and an association was considered statistically significant with P < 0.05. The t-test, Fisher’s exact test and the Mann-Whitney rank-sum test, and multivariable logistic regression were used for statistical analyses and to calculate odds ratios, respectively. Survival was determined for the period from the date of hospital admission to the date of the last search for death entries in the Social Security Index (date of search: December 31st, 2006). We obtained information (National Death Index, death certificates) on the causes of death for the deceased patients and censored all patients whose causes of death were not related to breast cancer. For logistic and Cox regression analyses, patients were stratified into no/yes mutation carriers and household income was stratified into three categories (< $15,000, between $15,000 and $60,000, > $60,000). Education was dichotomized into no high school degree and having a high school degree or above.

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**Author Contributions**

Conceived and designed the experiments: SA. Analyzed the data: AMS SA. Contributed reagents/materials/analysis tools: DNM THD BJB. Conceived and designed the experiments: SA. Analyzed the data: AMS.

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