

Mammary Cancer and Social Interactions: Identifying Multiple Environments That Regulate Gene Expression Throughout the Life Span

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Now that the human genome has been sequenced, along with those of major animal models, there is an urgent need to define those environments that interact with genes. The traditional view focuses on ways that gene products interact with the nuclear environment to regulate cell function, causing the physiologic changes, behaviors, and diseases manifest throughout development and aging. Although this view is essential, it is equally essential to understand the converse relationship, namely, to identify those environments at higher levels of organization that regulate the expression of specific genes. Given the vastness of this problem, one effective strategy is to start with a trait for which some of the genes have already been identified, such as malignant disease. In rats, social isolation and hypervigilance increase the incidence of mammary tumors, accelerate aging, and shorten the life span. We propose that similar environmental regulation of gene expression may underlie the disproportionately high mortality from premenopausal breast cancer of Blacks, a minority group that can experience high levels of loneliness and hypervigilance. Our goal is to identify which environments—social, psychological, hormonal, and cellular—regulate genetic mechanisms of mammary cancer risk as well as the specific times in the life span when they do so.

NEED FOR SPECIFYING MULTIPLE ENVIRONMENTS INTERACTING WITH GENES

The nature–nurture debate is ongoing, and its modern formation is: How do genes and the environment interact over time to produce a trait? The recent success of the Human Genome Project has been an important milestone. But the genetic information it provided demonstrates conclusively that sequencing the human genome marks only the first mile of a journey of cosmic proportions. Humans have fewer than 3×10^4 genes, less than those of a grain of rice (5×10^4) (Roest Crollius et al., 2000; Yu et al., 2002). How does the amount of information in the genome compare with that needed to account for human behavior? The human brain has 10^{10} neurons interconnected by 10^{15} synapses, with 10 synaptic events per synapse per second. Thus, during the half hour spent reading this article, a person's brain will experience at least 1.8×10^{20} synaptic events. The difference in the amount of information between genome and brain activity, let alone brain structure and the rest of the body, is on the order of 10^{16} . In miles, this is the diameter of a galaxy.

Obviously, the genome can only create a living being in interaction with its environment—the only other source of information in gene–environment interactions. A common misconception is to view this interaction as hierarchical—to view genes as the fundamental building blocks that interact with the nuclear environment to create cells, which interact to create structures or physiologic systems upward to individual psychology and social behaviors. But this view ignores the fact that a genome is not created *de novo*—It evolves through selection at the level of the individual, in dynamic interaction with its social and physical environment, enabling survival and

production of offspring that also survive to reproduce. This creates a selection pressure for genes that can or cannot be expressed in response to the demands of the environment. Therefore, the interaction between genes and environment is reciprocal—including not only genetic production of traits, but environmental regulation of genes throughout the life span. Indeed, only 25% of the variability in the life span of humans or baboons is attributed to genetic factors (Martin, Mahaney, Bronikowski, Dee Carey, Dyke, & Comuzzie, 2002; Skytthe et al., 2003).

Both the development of traits and the regulation of gene expression involve interactions not only with “the” environment but with many environments at multiple levels of analysis, ranging from the ecosystem, social systems, and the individual's immediate experiences, as well as environments within the individual set by physiologic systems, cell–cell interactions, structures within the cell's cytoplasm and the nucleus. These interactions also take place over multiple time spans: life in the moment (milliseconds to hours), life span development and aging (decades), cultural and ecologic systems (centuries), and evolutionary time. These multiple levels and time frames are all nested within one another, all potentially operating simultaneously. Thus, in principle, one cannot reduce the understanding of behavior or physical traits to genetic information. Nor can we know *a priori* which levels and time frames will be both necessary and sufficient for explaining the emergence of genetic traits.

One effective strategy for identifying environments that regulate gene expression is to begin with a genetic trait for which specific genes are known. Then we can ask: What is the

full range of environments that affect the expression of those genes? To illustrate these general principles, we present a hypothesis based on a synthesis of our collective research, which spans levels of analysis from ethnic populations to mechanisms for cell death and gene regulation. The trait in question is mammary cancer.

Mammary cancer develops after multiple environmental events, or “hits,” to the genome as well as inherited mutations. This involves a variety of tumor suppressor genes such as *BRCA1* and *BRCA2* as well as cooperative oncogenes such as *HER-2/neu* and *MYC* (Futreal et al., 1994; Lancaster et al., 1996; Miki, Katagiri, Kasumi, & Yoshimoto, 1996; Schmitt & Reis-Filho, 2003). Second, cells dividing unchecked by normal programmed cell death, termed “apoptosis,” are at greater risk for mutations producing cancer (Bai et al., 2001; Brash & Ponten, 1998; Soung et al., 2004; Sun, Li, & Sun, 1999). What environments might regulate these genetic mechanisms, and could they range from the cellular to the social?

DISPARITY OF BREAST CANCER MORTALITY BETWEEN BLACK AND WHITE WOMEN

Black women in the United States are twice as likely as Whites to die from breast cancer developed before menopause (Eley et al., 1994; English, Cleveland, & Barber, 2002; Hankey, Miller, Curtis, & Kosary, 1994; Shiao, Chen, Lehmann, Wu, & Correa, 1997). The difference in age dynamics is particularly striking, with young Black women experiencing a sharp increase between 30 and 44 years of age and then a relatively low rate thereafter that is relatively independent of age. In sharp contrast, breast cancer incidence in White women increases exponentially with age, with the greatest age-dependent frequency occurring after menopause. Black women are also more likely than Whites to have massive nonmalignant fibroadenomas, particularly in puberty (El-Tamer, Song, & Wait, 1999).

This striking health disparity likely arises from the reciprocal interplay of culture and biology that defines ethnicity and race. It is well established that human populations from a specific geographic origin can have lower genetic variance for some traits than do humans as a whole. Indigenous tribes of the island of Taiwan are genetically homogeneous within a tribe but diversified among them. Moreover, they have little genetic relationship to the Han of Mainland China (Lin et al., 2000). Likewise, the variant of a gene that increases the risk of prostate cancer (*CYP3A4*1B*) is found in 76% of Africans ($N=391$), 58% of African Americans ($N=261$), 7% of U.S. Caucasians ($N=573$), and 0% of Asians ($N=210$) (Rebeck, Jaffee, Walker, Wein, & Malkowicz, 1998; Zeigler-Johnson et al., 2002).

The disparity in mammary cancer may involve the *BRCA1* gene. *BRCA1*-associated breast cancers occur at an earlier average age (44 years) than sporadic breast cancers (Ford, Easton, Bishop, Narod, & Goldgar, 1994), and it has been observed that Blacks have a greater breast cancer incidence between 30 and 49 years than Whites (Adebamowo & Adegunle, 1999; Eley, 1994). Additionally, compared with noncarriers, *BRCA1*-associated breast cancers are characterized by higher than expected frequencies of medullary or atypical medullary carcinoma, poor differentiation (high tumor grade), aneuploidy, high S-phase fraction, and hormone receptor negativity (Phillips et al., 1999; Shiao et al., 1997). These

aggressive histologic features are also characteristic of breast cancer in Black women. These similarities suggest that alterations in *BRCA1* or related pathways might contribute to breast cancer in young Black women, although limited data are available from this population to evaluate the possibility.

Inherited germ-line mutations in *BRCA1* may reflect a genetic founder effect and create disparities in breast cancer among young Black women in the United States and Nigeria. Indeed, unique mutations in *BRCA1* (*1832del5*, *5296del4*, and *3883insA*) are found in Black but not White families (Gao, Neeuhausen, Cummings, Luce, & Olopade, 1997; Gao et al., 2000). These, as well as other genes with less penetrance, may be shared among all Black women of the African diaspora, particularly those originating in West Africa. Clinically, this is plausible, and genetic tests of tumors of Nigerian women are underway. Supporting this hypothesis is the clinical observation that Black and White women in Nigeria have a similar disparity in breast cancer and disease. Among young women, Blacks are most likely to develop breast cancer. The average age at diagnosis is 43 years, whereas Whites of European ancestry do not develop breast cancer until 60 years of age on average (Adebamowo & Adegunle, 1999; Ihekweba, 1992). Again, Black pubertal girls are more likely to develop rapidly growing fibroadenomas (Schneider, Laubenberger, Kommos, Madjar, Grone, & Langer, 1997).

However, inherited genetic mutations can be only one part of the disparity in breast cancer. Overall, only 5–10% of breast cancers have germ-line mutations as their origin (Kelsey, 1989; King, 1992; Skolnick & Cannon-Albright, 1992). Moreover, not all women with germ-line mutations of *BRCA1* develop breast cancer. Finally, there is no racial disparity in the frequency of germ-line (i.e., inherited) mutations (Gao et al., 1997, 2000). Environmental and physiologic factors regulate their penetrance. In these cases, psychosocial factors may increase the probability of somatic gene alterations, leading to breast cancers in women without a family history.

Many health behaviors, diets, and environmental factors modulate breast cancer risk, both in humans and in animal models. These include, among others: obesity, lack of exercise, high dietary fat, and exposure to carcinogens (Bernstein, 2002; Kim, 2002; Salih & Fentiman, 2001). In addition, there are disparate availability and access to health care systems, which can increase mortality from breast cancer (Coughlin, Thompson, Hall, Logan, & Uhler, 2002). Many of these factors, however, have been insufficient to explain the extreme health disparity in breast cancer between Blacks and Whites, particularly in young women (Jatoi, Becher, & Leake, 2003; Marie Swanson, Haslam, & Azzouz, 2003). Moreover, cancer is a multistep process, and there are undoubtedly a host of ways that the environment and behavior can increase the series of mutations necessary for the initiation and growth of mammary cancer.

In this article, we identify three environments at different levels of analysis: social isolation, hypervigilance (constant alertness for potential danger), and ovarian function throughout the life span. Each has a demonstrated disparity between Blacks and Whites, and each, in animal models, increases risk factors for mammary cancer. We hypothesize that it is the interaction of each of these environments that increases mammary cancer risk. The interaction changes cellular and genetic functions, such as preventing programmed cell death in breast tissue, and

creates somatic alterations in tumor-regulating genes, such as hypermethylation of *BRCA1*.

PSYCHOSOCIAL PREDISEASE PATHWAYS AND RISK FACTORS

In seminal studies, House and colleagues (House, Landis, & Umberson, 1988; House, Robbins, & Metzner, 1982) demonstrated that social isolation and felt loneliness (i.e., perceived and self-reported) were correlated with high rates of all-cause mortality. The question now becomes: What are the myriad routes by which the social environment “gets under the skin” and exacerbates disease (Harrington, 2000; Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002)? Cacioppo and colleagues (Cacioppo et al., 2002a, 2002b; Hawkey, Bureson, Berntson, & Cacioppo, 2003) have identified some possible routes. Felt loneliness is associated with increased total peripheral resistance (i.e., activation of the sympathetic nervous system), disrupted sleep, and altered neuroendocrine function. Importantly, neither the frequency of actually being alone nor health behaviors differed between the lonely and nonlonely groups, providing strong evidence that it is felt loneliness, rather than being alone, that is the important component of a predisease pathway for cardiovascular disease.

There is emerging evidence that Blacks, as a group, are socially isolated and lonely. In *Heat Wave: A Social Autopsy of Disaster in Chicago*, Klinenberg (2002) determined that elderly poor Blacks were the group most likely to die during a recent Chicago heat wave. Hispanics with the same socioeconomic profiles were much less likely to die, because their social structures provided relationships with people who looked after them, whereas poor Blacks were the most isolated. Also, Patterson (1997) in his book titled *The Ordeal of Integration: Progress and Resentment in America's "Racial" Crisis*, while discussing marriage patterns, suggests that Blacks might be the “loneliest people in the world.”

Other psychological environments may also provide a route “under the skin.” We anticipate that high rates of crime and dilapidation of housing in some Black neighborhoods on the South Side of Chicago contribute to health disparities in a number of ways. First, violent crime decreases social trust and increases the expectation that others will take advantage of you (Wilkinson, 1999). And, those who do not trust others are more likely to live alone. Crime likewise contributes to a threatening environment and the need for hypervigilance for potential threats, especially if one lives alone in unsafe housing (Sampson, Raudenbush, & Earls, 1997). Black women might also be more hypervigilant, by virtue of the disproportionate burden of economic uncertainty that they share (Steele & Sherman, 1999; Wilson, 1996). Living as a minority itself can also entail a daily accumulation of stressors, loneliness, and social isolation (Williams, Neighbors, & Jackson, 2003).

Finally, the problems of an extended family environment fall disproportionately on many middle-aged women, who are at risk for breast cancer, such as the increasing number of Black women who are faced with parenting their grandchildren (Waite, 2000). For many women, this may be exacerbated in the household context, with its associated daily hassles and demands. In contrast, social supports are certainly available in Black communities, particularly attending church, which is associated with lower mortality from cancer (Hummer, Rogers, Nam, & Ellison, 1999). As Waite and Hughes argue (Waite,

2000), the key factor in determining hypervigilance and social isolation may well not be the actual burdens, threats, and stressors present in the environment, but rather the fact that they outweigh the available supports.

Such social and psychological disparities between Blacks and Whites are not unique to Americans or even to the contrast between Blacks and Whites. In Ibadan, Oyo State, Nigeria, psychosocial disparities among Nigerian women appear to be as marked as those between some Black and White women in America. Ibadan has been a major city of the Yoruba people for hundreds of years, and women of families who have lived there for generations enjoy considerable family and financial supports, as do the highly educated élite. In sharp contrast, other women are caught in social conflicts caused by dramatic rapid modernization. This is particularly true for women transplanted from rural society to the city in response to a rapidly globalizing economy, creating conflicts between urban and rural practices and values (Arason, Barkardottir, & Egisson, 1993). It is among these women that social isolation and vigilance are likely to be highest. Rural women who move to the city to find better markets for their small-scale commerce (e.g., Ijesa osomaalo, or cloth traders) often lose the social supports typical of their rural community (van't Veer et al., 2002). This is particularly so for women of non-Yoruba tribal groups such as Igbo or Housa.

Such disparities in social support have been documented in South Africa; urban Blacks experience lower social support than their rural counterparts (Mboya, 2000). In rural towns as well, commercialization of the economy has changed the traditional system of economic supports flowing along family lines (Guyer, 1990). Even when members of the same family lineage move to Ibadan, few live in the same urban compounds or housing complexes (Arason et al., 1993), eroding economic and social supports enjoyed by extended family living together in a rural town. In becoming urbanized, women may also lose the highly valued communal quality of rural life (Gugler & Flanagan, 1978). Finally, Yoruba women traditionally expect to be financially independent, yet the urban economy amplifies the disparity of women's earning capacity (Zeitlin, Megawangi, Kramer, Colletta, Babatunde, & Garnabm, 1995). Women have more difficulty than do men finding jobs that offset the high cost of urban living. Often, jobs are not close to their homes and childcare support is scarce (van't Veer et al., 2002). Thus, we hypothesize that it is these Nigerian women in particular, not all Black Nigerian women, that are at risk for early aggressive breast cancer.

DISRUPTION OF DNA METHYLATION IN SPORADIC BREAST CANCER

Most mammary tumors do not result from a known gene mutation inherited via the germ cells (sperm or egg). Nor do they follow a pattern of family inheritance, or clustering; they are thus termed “sporadic”. Analysis of sporadic breast and ovarian tumors has revealed a very low frequency of *BRCA1* and *BRCA2* heritable mutations in such tumors (Foster et al., 1996; Futreal et al., 1994; Hosking et al., 1995; Lancaster et al., 1996; Merajver et al., 1995; Miki et al., 1996; Takahashi et al., 1996; Teng et al., 1996). Despite the lack of heritable mutations, several lines of evidence implicate malfunction of *BRCA1* and *BRCA2* genes in sporadic tumors. Such malfunction can be

acquired during a woman's lifetime through a variety of mutagenic environmental events, particularly random mutations during failure of apoptosis (cell death), as is discussed later. More interestingly, malignancy is associated with hypermethylation of tumor-suppressor genes, which "silences" it or its promoter, and hypomethylation of tumor-enhancing genes.

Disruption of the normal DNA methylation patterns is an established common hallmark of human cancer cells. In a healthy cell, the DNA methylation patterns are conserved through cell divisions, allowing the expression of the particular set of cellular genes necessary for that cell type and blocking the expression of exogenously inserted sequences (Esteller et al., 2001). Cancer cells often exhibit the dual phenomena of global hypomethylation accompanied by hypermethylation of several small CpG islands, an area, typically near promoters, with a high frequency of the C-G sequence, connected by a phosphor-diester bond (Jones & Laird, 1999). The aberrant methylation of the CpG island located in the 5'-promoter region of several tumor suppressor genes such as *hMLH1*, *VHL*, *CDH1*, *p16INK4a*, and *APC* shuts down the expression of these contiguous genes.

Even though it is not mutated, *BRCA1* is likely involved in sporadic tumors. Its high frequency of loss of heterozygosity in sporadic tumors (Cropp et al., 1994; Foulkes, Black, Stamp, Solomon, & Trowsdale, 1993; Lindblom, Skoog, Andersen, Rotstein, Nordenskjold, & Larsson, 1993), decreased activity in sporadic tumors once they become invasive (Thompson, Jensen, Obermiller, Page, & Holt, 1995), increased proliferation of mammary epithelium with antisense oligonucleotides to *BRCA1* (Rao, Shao, Ahmad, & Reddy, 1996), and the decreased tumorigenicity of cell lines derived from sporadic tumors with the introduction of a normal copy of the *BRCA1* gene (Holt et al., 1996) all indicate involvement in sporadic breast cancer. For *BRCA2*, the loss of heterozygosity observed in 30–40% of sporadic primary breast cancers suggests it, too, is involved in sporadic cases (Cleton-Jansen et al., 1995).

Promoter hypermethylation has recently been suggested as mechanism for *BRCA1* inactivation in sporadic breast and ovarian cancers ranging in frequency from 11% to 20% (Biano, Hussey, & Dobrovic, 1999; Catteau, Harris, Xu, & Solomon, 1999; Dobrovic & Simpfendorfer, 1997). In contrast, *BRCA2* seems to undergo hypomethylation and is frequently overexpressed in tumor cells (Thyaga Rajan & Felten, 2002), hence our focus on *BRCA1* promoter methylation as a candidate for environmental regulation of breast cancer. The challenge, then, is to establish physiologic mechanisms through which psychosocial environments such as social isolation and hypervigilance can alter methylation of specific cancer genes and their promoters or increase the probability of spontaneous mutations through hyperproliferation of cells.

A RODENT MODEL FOR ENVIRONMENTAL REGULATION OF BREAST CANCER GENETICS

Female Norway rats are a powerful animal model for studying how social isolation and hypervigilance regulate the biological environments that produce mammary cancers. Females in this species are highly social, living and sleeping together in a group within the burrow system. They even cooperate in rearing their young (Blumberg, Mennella, Moltz, & McClintock, 1992). Thus, the genetic, hormonal, and immunologic systems for

copied with disease co-evolved in the physiologic environment associated with social living. When the female rats' social system is disrupted by isolation and loss of supportive social interactions, they develop mammary carcinomas and hyperplasia at four times the rate of their same aged counterparts living in a group (Figure 1). This occurs at 14 months of age, equivalent to 35–45 years of age in humans (Minino & Smith, 2001) and similar to the peak of early onset of premenopausal breast cancer observed in Black women (English et al., 2002). By 17 months of age, the mammary cancer burden in isolated females is 16 times that of those living in groups (Hermes & McClintock, submitted). In contrast, group-living female rats do not develop a high rate of mammary tumors until late in their life span, at 23 months (see Figure 1), which is equivalent to 60–70 years of age in humans, similar to the pattern observed in postmenopausal White women.

In addition, social isolation makes female rats hypervigilant, manifest behaviorally by failure to explore novel but benign environments (termed "neophobia"), a frozen posture with erect fur, and reduced ability to forage efficiently for food, even when the environment is familiar (S. A. Cavigelli, G. Hermes, and M. K. McClintock, unpublished data). In male rats, even those living with their brothers, vigilant neophobic animals develop and die from tumors at a younger age than do calm neophilic rats, which find new environments a challenge rather than a threat (Cavigelli & McClintock, 2003). Neophobic males and socially isolated females both have prolonged secretion of glucocorticoids in response to the stressors of everyday life (Cavigelli & McClintock, 2003; G. Hermes & M. K. McClintock, submitted).

In this rodent model, the disparity in early fatal mammary tumors cannot be genetic, because the animals are randomly assigned to their respective social conditions, balanced for family of origin. Moreover, obesity and exercise levels do not explain the disparity. In addition, they all live in the same laboratory room where they can see, smell, and hear each other, with the same food, light, temperature, and animal care protocols. Thus, in the animal model, it is the psychosocial environment that is regulating the cellular and genetic processes producing tumors.

Our rat model focuses on tumor development in the context of natural physiology, particularly the dynamics of undisturbed ovarian function throughout the life span and responses of the adrenal axis to mild stressors during a protected life in a laboratory colony. Thus, it extends the scope and generality of *in vivo* models of cancer, beyond artificially manipulating hormonal states through chronic implants of different hormones. Here we have the opportunity to consider the dynamics of natural variation in ovarian and adrenal states as mechanisms of mammary development and tumorigenesis.

PUBERTY AND ADULT OVARIAN FUNCTION

In humans, increased risk for breast cancer is associated with early puberty. It is also commonly reported that puberty occurs earlier in Black girls than in Whites. In these studies, puberty onset is measured by breast bud development (Tanner Stage B2) (Wu, Mendola, & Buck, 2002). Strikingly, there is a much smaller disparity in age at menarche, maturation of ovarian function, between Black and White girls (Cameron, Grieve, Kruger, & Leschner, 1993; Herman-Giddens et al., 1997; Lee, Guo, & Kulin, 2001; Morrison, Barton, Biro, Sprecher, Falkner, & Obarzanek, 1994; Mul, Fredriks, van Buren, Oostdijk,

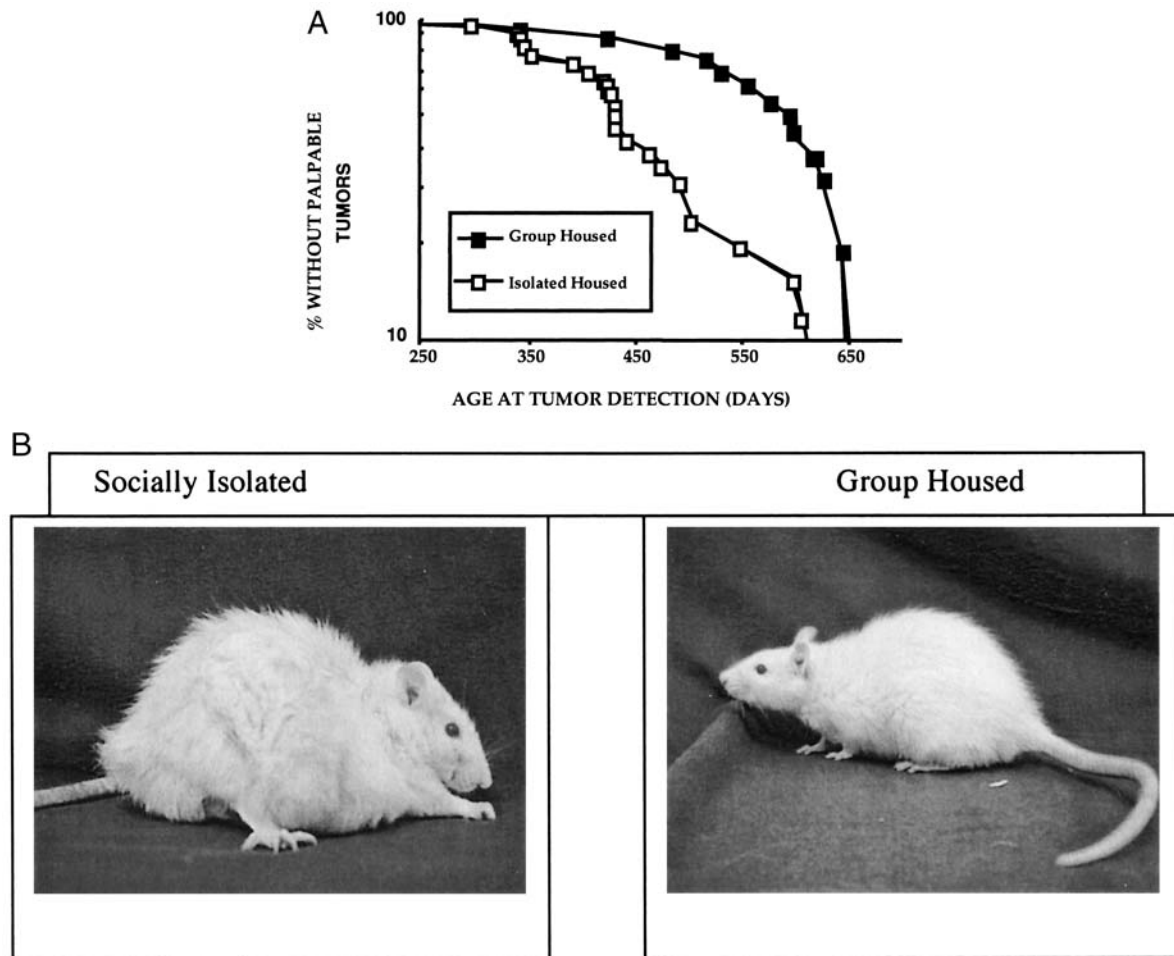


Figure 1. **A**, Socially isolated female rats develop palpable mammary tumors at a younger age than do highly inbred animals randomly assigned to live in groups. **B**, Both the socially isolated and the group-housed rats are 790 days of age. The socially isolated animal has multiple mammary tumors and is emaciated.

Verloove-Vanhorick, & Wit, 2001). Therefore, a crucial pubertal risk factor may not be early puberty per se, but instead prolonged breast development prior to the onset of menarche and afterward during cyclic exposure to ovarian steroids.

During the interval between breast development and menarche, the hormonal milieu differs markedly from that once regular ovarian cycles are established. Sex hormone binding globulin is variable (Bedecarras, Gryngarten, Ayuso, Escobar, Bergada, & Campo, 1998), growth hormone is higher than it is after menarche, with higher basal and nighttime levels and more high pulses (Neville, McFadden, & Forsyth, 2002; Wennink, Delemarre-van de Waal, Schoemaker, Blaauw, & van den Braken, 1991), and estradiol release is pulsatile, rather than continuous, particularly at night (Mitamura, Yano, Suzuki, Ito, Makita, & Okuna, 2000). Each of these hormone dynamics affects mammary development and the development of fibroadenomas in Black adolescents (Naidu, Thomson, & Nirmul, 1989) as well as risk for mammary cancer. It is intriguing that the Black African populations in which these pubertal disparities do not occur are also those with relatively high social supports and networks: that is, rural, not urban, girls in South Africa (Cameron & Wright, 1990; Cameron et al.,

1993) and upper middle class urban schoolgirls in Nigeria (Fakeye & Fagbule, 1990).

If exposure to ovarian steroids after menarche is an environmental risk factor, it is likely that the temporal dynamics of spontaneous ovarian cycles are critical. It is well established that it is the dynamic change in hormones that regulates other endocrine systems (Larsen, Kronenberg, Melmed, & Polonsky, 2002). A rapid increase in estradiol, not its level, triggers the luteinizing hormone surge. It is the rise in corticosterone, not its level, that regulates negative feedback on the hypothalamic-pituitary-adrenal axis.

In both humans and rats, social interactions, mediated by pheromones, change ovarian function. Specifically, they regulate the timing of the preovulatory surge of luteinizing hormone, thereby affecting the duration of unopposed estrogen, prolactin pulses, and corticosterone levels (McClintock, 1978, 1983, 1984; Stern & McClintock, 1998). Social isolation disrupts this social regulation of ovarian function. It also changes independent neuroendocrine mechanisms underlying reproductive behaviors (Gans & McClintock, 1993; Gans, Stamper, Butler, & McClintock, 1995) and undoubtedly alters the hormonal milieu of mammary tissue during puberty and adulthood.

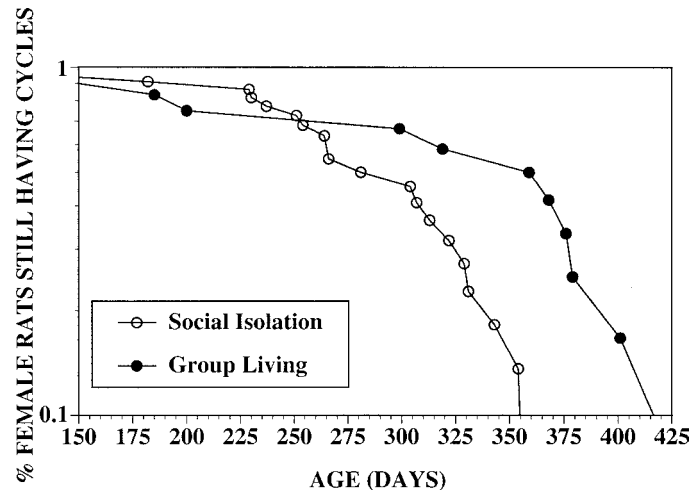


Figure 2. Social isolation accelerates termination of regular estrous cycles and onset of reproductive senescence.

There is a paradox, however, later in the life span when tumors are developing, social isolation accelerates reproductive aging, and isolated females at the age of tumorigenesis are no longer having spontaneous ovarian cycles (Figure 2) (G. Hermes & M. K. McClintock, submitted; LeFevre & McClintock, 1988, 1991). They have reached reproductive senescence and are producing only low tonic levels of estrogen. The strong role of estrogen receptors in some mammary cancers predicts the opposite pattern in rats as well as humans. This points to yet another hormonal environment created by social isolation that may accelerate tumor growth late in the life span.

STRESS HORMONES AND INHIBITION OF PROGRAMMED CELL DEATH

We hypothesize that a reduction in programmed cell death combined with normal proliferation of mammary epithelial cells causes hyperplasia of mammary epithelial cells and might contribute to the increased incidence of fibroadenomas as well as additional mutations leading to breast cancer. Programmed cell death can be reduced by activation of glucocorticoid receptors (GRs) in tissue (receptors that bind glucose secreted by the adrenal). Indeed, social isolation prolongs elevated levels of glucocorticoids in response to the stressors of everyday life (G. Hermes & M. K. McClintock, submitted). Thus, it appears to accelerate aging of the hypothalamic–pituitary–adrenal axis and hippocampus (Sapolsky, Krey, & McEwen, 1986). The failure of socially isolated rats to rapidly return to a baseline level of corticosterone is postulated to result in chronic GR activation, thereby inhibiting apoptosis of the ductal epithelium and increasing risk for spontaneous mutations causing mammary cancer.

The identification of antiapoptotic signaling mechanisms has provided an experimental framework for understanding how premalignant and malignant cells can escape environmental signals that would normally initiate programmed cell death. The critical importance of identifying survival pathways relevant to specific cell types is highlighted by studies that have associated activation of individual antiapoptotic pathways with treatment failure in some tumor cell types and not others (Schmitt & Lowe, 1999).

In mammary epithelial cells, Conzen and colleagues (Moran, Gray, Mikosz, & Conzen, 2000) recently defined a novel GR-mediated survival mechanism that is induced by prolonged exposure to physiologic concentrations of glucocorticoid and inhibited by GR-specific antagonists (antiglucocorticoids). GR antagonists have been shown previously to interfere with glucocorticoid function by a two-step process: competitive inhibition of glucocorticoid receptor binding and competition of the antagonist-bound receptor with that of the glucocorticoid-bound receptor on DNA response elements within target gene promoters (Wagner et al., 1999). Because active antiglucocorticoid compounds inhibit the transactivation (and possibly the repression) of GR-specific target genes and also block GR-mediated survival signaling, we hypothesize that the mechanism through which the GR induces survival requires the induction and/or repression of cell type-specific “survival genes” (Mikosz, Brickley, Sharkey, Moran, & Conzen, 2001).

Glucocorticoids are well known for their anti-inflammatory and immunosuppressive properties as well as for their essential role in embryonic development. The majority of glucocorticoids’ properties are thought to be a consequence of the ability of the activated GR to act as a transcription factor, either through a direct DNA binding–dependent mechanism or through cross-talk and often interference with other transcription factors such as AP1, STAT5, and nuclear factor- κ B (Dumont et al., 1998). In addition, so-called “nongenomic” effects may play a role in the rapid effects of glucocorticoids on cell signaling (Borski, 2000).

Although the GR is expressed ubiquitously in normal human mammary epithelium as well as breast cancers, the role of the GR in breast cancer biology and mammary gland development has, until recently, received relatively little attention. Indeed, 100 articles have been published on the role of GRs in mammary tumor biology in contrast to >11,000 on estrogen receptors (2004 PubMed search with key terms of “breast cancer” and “glucocorticoid receptor” or “estrogen receptor”).

In vitro, a physiologic concentration of hydrocortisone (10^{-6} M) has long been added to the mixture of survival factors required for successful epithelial cell growth in serum-free conditions; furthermore, the importance of glucocorticoids for

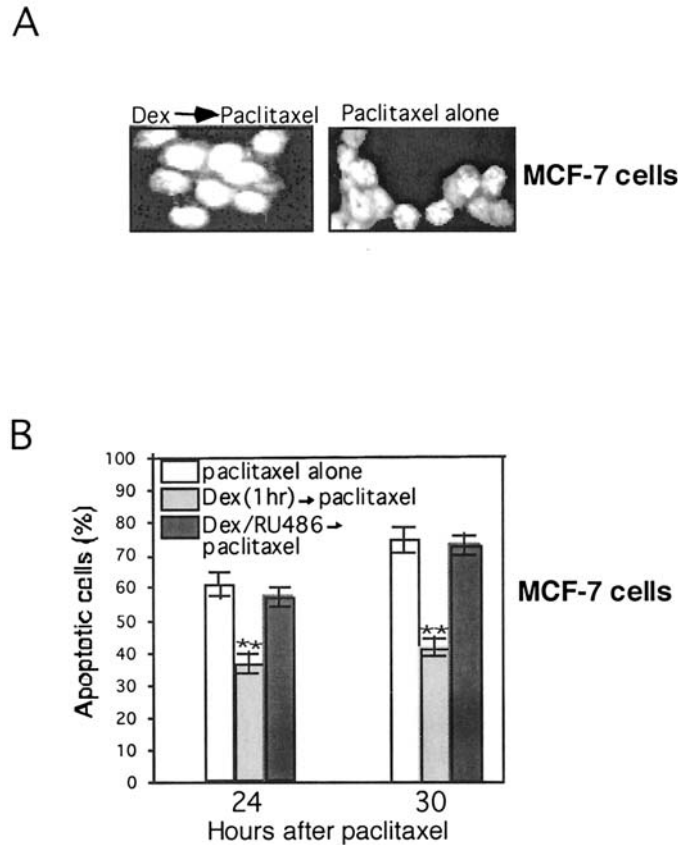


Figure 3. **A**, Apoptosis in MCF-7 breast cancer cells is inhibited by pretreatment with physiologic concentrations of dexamethasone of 10^{-6} M (a glucocorticoid-like drug). The panel on the right shows DAPI-stained apoptotic cells with condensed chromatin in the nucleus, after treatment with paclitaxel chemotherapy; the panel on the left shows normal DAPI-stained cells with normal nuclei when treated with dexamethasone prior to chemotherapy. **B**, Glucocorticoids (dexamethasone) protect MCF-7 cells from apoptosis, while dexamethasone plus concomitant RU486 (10^{-7} M) treatment (adding a glucocorticoid receptor blocker) reverses the protection from apoptosis afforded by glucocorticoid receptor activation.

optimal plating efficiency of mammary epithelial cells has suggested a possible role in cell survival (Hammond, Ham, & Stampfer, 1984). In vivo, systemic glucocorticoid treatment has been observed to prevent mammary gland involution and concomitant mammary epithelial cell apoptosis in the glands of lactating mice weaning their young (Lund et al., 1996). Moreover, a GR DNA binding mutant knock-in (GRdim) has recently been shown to have defects in mammary gland development, suggesting a direct role for GR transcriptional activation in mammary epithelial cell proliferation and possibly apoptosis (Reichardt et al., 2001).

What is the intracellular pathway transducing the glucocorticoid hormonal environment, triggered by psychosocial factors, into the cellular environment regulating cell death? One such important downstream effector of glucocorticoids' antiapoptotic function, in both mammary epithelial cells and breast cancer cell lines subjected to growth factor deprivation-induced apoptosis, is serum- and glucocorticoid-inducible kinase (SGK-1) (Mikosz et al., 2001). SGK-1 is a novel serine-threonine kinase that was identified as a transcriptional target of glucocorticoid in a rat mammary tumor cell line (Webster, Goya, Ge, Maiyar, & Firestone, 1993) and was initially studied as a potential mammary cell cycle regulatory protein (Buse, Tran, Luther, Phu,

Aponte, & Firestone, 1999) in rat tumor cells. Recently, two additional isoforms of SGK have been identified: SGK-2 and SGK-3. However, neither of these isoforms appears to be regulated transcriptionally (Casamayor, Torrance, Kobayashi, Thorner, & Alessi, 1999).

Both GR activation and SGK-1 expression can also inhibit apoptosis in human breast cancer cell lines subjected to paclitaxel- or doxorubicin-induced apoptosis at clinically relevant concentrations (Wu, Chaudhuri, Brickley, Pang, Karison, & Conzen, 2004) (Figure 3). The administration of RU486, a GR blocker, fully reinstates cell death in response to these chemotherapeutic agents, demonstrating the central role of GRs in regulating apoptosis (see Figure 3B).

Thus, we anticipate that the prolonged secretion of glucocorticoids by socially isolated female rats, following everyday stressors of laboratory life, will be associated with GR expression in mammary ductal epithelium and stroma. Gene array analysis can be used to compare GR target gene expression in socially isolated and group-housed animals. This will be a direct test of the hypothesis that persistent stress hormone signaling through GR activation causes expression of GR targets that contribute to the development of benign and malignant mammary tumors.

IMPLICATIONS

We have used the context of health disparities between Black and White women to identify multiple environments that may regulate gene expression and increase the incidence of aggressive premenopausal breast cancer. Here, differences in social and psychological environments are writ large—large enough to test hypotheses about environmental regulation of gene expression. But, once established, the multiple environments regulating gene function to produce this health disparity will likely generalize broadly to men and women of all ethnic groups. There is certainly individual variation in levels of felt loneliness and hypervigilance in the face of threats, both real and perceived. And this individual variation in psychosocial environments likely contributes to methylation of cancer genes that are part of the ontogeny of malignant disease and mutations increased by the failure of tissue-specific programmed cell death.

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REFERENCES

- Adebamowo, C. A., & Adekunle, O. O. (1999). Case-controlled study of the epidemiological risk factors for breast cancer in Nigeria. *British Journal of Surgery*, *86*, 665–668.
- Arason, A., Barkardottir, R. B., & Egisson, V. (1993). Linkage analysis of chromosome 17q markers and breast-ovarian cancer in Icelandic families and possible relationship to prostatic cancer. *American Journal of Human Genetics*, *52*, 711–717.
- Bai, M., Agnantis, N., Kamina, S., Demou, A., Zagorianakou, P., Katsaraki, A., et al. (2001). In vivo cell kinetics in breast carcinogenesis. *Breast Cancer Research*, *3*, 276–283.
- Bedecarras, P., Gryngarten, M., Ayuso, S., Escobar, M. E., Bergada, C., & Campo, S. (1998). Characterization of serum SHBG isoforms in prepubertal and pubertal girls. *Clinical Endocrinology (Oxford)*, *49*, 603–608.
- Bernstein, L. (2002). Epidemiology of endocrine-related risk factors for breast cancer. *Journal of Mammary Gland Biology and Neoplasia*, *7*, 3–15.
- Biano, T., Hussey, D., & Dobrovic, A. (1999). Methylation-sensitive, single-strand conformation analysis (MS-SSCA): A rapid method to screen for and analyze methylation. *Human Mutation*, *14*, 289–293.
- Blumberg, M., Mennella, J., Moltz, H., & McClintock, M. K. (1992). Facultative sex ratio adjustment in Norway rats: Litters born asynchronously are female biased. *Behavioral Ecology and Sociobiology*, *31*, 401–408.
- Borski, R. J. (2000). Nongenomic membrane actions of glucocorticoids in vertebrates. *Trends in Endocrinology and Metabolism*, *11*, 427–436.
- Brash, D., & Ponten, J. (1998). Skin precancer. *Cancer Surveys*, *32*, 69–113.
- Buse, P., Tran, S. H., Luther, E., Phu, P. T., Aponte, G. W., & Firestone, G. L. (1999). Cell cycle and hormonal control of nuclear-cytoplasmic localization of the serum- and glucocorticoid-inducible protein kinase, Sgk, in mammary tumor cells. A novel convergence point of anti-proliferative and proliferative cell signaling pathways. *Journal of Biological Chemistry*, *274*, 7253–7263.
- Cacioppo, J. T., Hawkey, L. C., Bernston, G. G., Ernst, J. M., Gibbs, A. C., Stickgold, R., et al. (2002a). Lonely days invade the nights: Social modulation of sleep efficiency. *Psychological Science*, *13*, 384–387.
- Cacioppo, J. T., Hawkey, L. C., Crawford, L. E., Ernst, J. M., Bursleson, M. H., Kowalewski, R. B., et al. (2002b). Loneliness and health: Potential mechanisms. *Psychosomatic Medicine*, *64*, 407–417.
- Cameron, N., Grieve, C. A., Kruger, A., & Leschner, K. F. (1993). Secondary sexual development in rural and urban South African black children. *Annals of Human Biology*, *20*, 583–593.
- Cameron, N., & Wright, C. A. (1990). The start of breast development and age at menarche in South African black females. *South African Medical Journal*, *78*, 536–539.
- Casamayor, A., Torrance, P. D., Kobayashi, T., Thorner, J., & Alessi, D. R. (1999). Functional counterparts of mammalian protein kinases PDK1 and SGK in budding yeast. *Current Biology*, *9*, 186–197.
- Catteau, A., Harris, W. H., Xu, C. C., & Solomon, E. (1999). Methylation of the *BRCA1* promoter region in sporadic breast and ovarian cancer: Correlation with disease characteristics. *Oncogene*, *18*, 1957–1965.
- Cavigelli, S. A., & McClintock, M. K. (2003). Fear of novelty in infant rats predicts adult corticosterone dynamics and an early death. *Proceedings of the National Academy of Sciences*, *100*, 16131–16136.
- Cleton-Jansen, A. M., Collins, N., Lakhani, S. R., Weissenbach, J., Devilee, P., Cornelisse, C. J., et al. (1995). Loss of heterozygosity in sporadic breast tumours at the *BRCA2* locus on chromosome 13q12–q13. *British Journal of Cancer*, *72*, 12414.
- Coughlin, S. S., Thompson, T. D., Hall, H. I., Logan, P., & Uhler, R. J. (2002). Breast and cervical carcinoma screening practices among women in rural and non-rural areas of the United States, 1998–1999. *Cancer*, *94*, 2801–2812.
- Cropp, C. S., Nevanlinna, H. A., Pyrhonen, S., Stenman, U. H., Salmikanaas, P., Albertsen, H., et al. (1994). Evidence for involvement of *BRCA1* in sporadic breast carcinomas. *Cancer Research*, *54*, 2548–2551.
- Dobrovic, A., & Simpfendorfer, D. (1997). Methylation of the *BRCA1* gene in sporadic breast cancer. *Cancer Research*, *57*, 3347–3350.
- Dumont, A., Hehner, S. P., Schmitz, M. L., Gustafsson, J. A., Liden, J., Okret, A., et al. (1998). Cross-talk between steroids and NF-kappa B: What language? *Trends in Biochemical Sciences*, *23*, 233–235.
- Eley, J. W., Hill, H. A., Chen, V. S., Austin, D. F., Wesley, M. N., Muss, H. B., et al. (1994). Racial differences in survival from breast cancer. Results of the National Cancer Institute Black/White Cancer Survival Study. *Journal of the American Medical Association*, *272*, 947–954.
- El-Tamer, M. B., Song, M., & Wait, R. B. (1999). Breast masses in African American teenage girls. *Journal of Pediatric Surgery*, *34*, 1401–1404.
- English, W. P., Cleveland, K. E., & Barber, W. H. (2002). There is no difference in survival between African-American and white women with breast cancer. *American Journal of Surgery*, *68*, 594–597.
- Esteller, M., Fraga, M. F., Guo, M., Garcia-Foncillas, J., Hedenfal, I., Godwin, A. K., et al. (2001). DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis. *Human Molecular Genetics*, *10*, 3001–3007.
- Fakeye, O., & Fagbule, D. (1990). Age and anthropometric status of Nigerian girls at puberty: Implication for the introduction of sex education into secondary schools. *West African Journal of Medicine*, *9*, 226–231.
- Ford, D., Easton, D., Bishop, D., Narod, S., & Goldgar, D. (1994). Risks of cancer in *BRCA1*-mutation carriers. Breast Cancer Linkage Consortium. *Lancet*, *343*, 692–695.
- Foster, K. A., Harrington, P., Kerr, J., Russell, P., DiCioccio, R. A., Scott, I. V., et al. (1996). Somatic and germ line mutations of the *BRCA2* gene in sporadic ovarian cancer. *Cancer Research*, *56*, 3622–3625.
- Foulkes, W. D., Black, D. M., Stamp, G. W., Solomon, E., & Trowsdale, J. (1993). Very frequent loss of heterozygosity throughout chromosome 17 in sporadic ovarian carcinoma. *International Journal of Cancer*, *54*, 220–225.
- Futreal, P. A., Lu, Q., Shattuck, E. D., Cochran, C., Harshman, K., Tavtigian, S., et al. (1994). *BRCA1* mutations in primary breast cancer and ovarian carcinomas. *Science*, *266*, 120–122.
- Gans, S., & McClintock, M. K. (1993). Individual differences in timing of the preovulatory LH surge are predicted by lordosis reflex intensity. *Hormones and Behavior*, *27*, 403–417.
- Gans, S., Stamper, J. L., Butler, T., & McClintock, M. K. (1995). Endocrine basis for two types of individual differences in lordosis reflex intensity. *Hormones and Behavior*, *29*, 357–391.
- Gao, Q., Neuhuhausen, S., Cummings, S., Luce, M., & Olopade, O. (1997). Recurrent germ-line *BRCA1* mutations in extended African American families with early-onset breast cancer. *American Journal of Human Genetics*, *60*, 1233–1236.
- Gao, Q., Tomlison, G., Das, S., Cummings, S., Sveen, L., Fackenthal, J., et al. (2000). Prevalence of *BRCA1* and *BRCA2* mutations among

- clinic-based African American families with breast cancer. *Human Genetics*, 107, 186–191.
- Gugler, J., & Flanagan, W. (1978). *Urbanization and social change in West Africa*. New York: Cambridge University Press.
- Guyer, J. (1990). *Changing nuptiality in a Nigerian community: Observations from the Field. Working Papers in African Studies* (vol. 146). Boston: African Studies Center, Boston University.
- Hammond, S. L., Ham, R. G., & Stampfer, M. R. (1984). Serum-free growth of human mammary epithelial cells: Rapid clonal growth in defined medium and extended serial passage with pituitary extract. *Proceedings of the National Academy of Sciences*, 81, 5435–5439.
- Hankey, B. F., Miller, B., Curtis, R., & Kosary, C. (1994). Trends in breast cancer in younger women in contrast to older women. *Journal of the National Cancer Institute, Monographs* 16, 7–14.
- Harrington, A. (2000). The whiteness of lies: Swallowing the placebo effect. *Cerebrum: the Dana Forum on Brain Science*, 2, 1.
- Hawkey, L. C., Burleson, M. H., Bertson, G. G., & Cacioppo, J. T. (2003). Loneliness in everyday life: Cardiovascular activity, psychosocial context, and health behaviors. *Journal of Personality and Social Psychology*, 85, 105–120.
- Herman-Giddens, M. E., Slora, E. J., Wasserman, R. C., Bourdony, C. J., Bhapkar, M. V., Koch, G. G., et al. (1997). Secondary sexual characteristics and menses in young girls seen in office practice: A study from the Pediatric Research in Office Settings network. *Pediatrics*, 99, 505–512.
- Hermes, G., & McClintock, M. K. (submitted). Social isolation accelerates puberty yet amplifies stress and increases mammary tumors. *Proceedings of the National Academy of Sciences*.
- Holt, J. T., Thompson, M. E., Szabo, C., Robinson, B. C., Arteaga, C. L., King, M. C., et al. (1996). Growth retardation and tumor inhibition by *BRCA1*. *Nature Genetics*, 12, 298–302. Erratum (1998): *Nature Genetics*, 19, 102.
- Hosking, L., Trowsdale, M. E., Szabo, C., Robinson, B. C., Arteaga, C. L., Kong, M. C., et al. (1995). A somatic *BRCA1* mutation in an ovarian tumour. *Nature Genetics*, 9, 343–344.
- House, J. S., Landis, K. R., & Umberson, D. (1988). Social relationships and health. *Science*, 241, 540–545.
- House, J. S., Robbins, C., & Metzner, H. L. (1982). The association of social relationships and activities with mortality: Prospective evidence from the Tecumseh Community Health Study. *American Journal of Epidemiology*, 116, 123–140.
- Hummer, R. A., Rogers, R. G., Nam, C. B., & Ellison, C. G. (1999). Religious involvement and U.S. adult mortality. *Demography*, 36, 273–285.
- Ihekwaba, F. N. (1992). Breast cancer in Nigerian women. *British Journal of Surgery*, 79, 771–775.
- Jatoi, I., Becher, H., & Leake, C. (2003). Widening disparity in survival between white and African-American patients with breast carcinoma treated in the U.S. Department of Defense Healthcare System. *Cancer*, 98, 894–899.
- Jones, P. A., & Laird, P. W. (1999). Cancer epigenetics comes of age. *Nature Genetics*, 21, 163–167.
- Kelsey, J. L. (1989). A review of the epidemiology of human breast cancer. *Epidemiologic Reviews*, 1, 74–109.
- Kiecolt-Glaser, J. K., McGuire, L., Robles, T. F., & Glaser, R. (2002). Emotions, morbidity, and mortality: New perspectives from psychoneuroimmunology. *Annual Review of Psychology*, 53, 83–107.
- Kim, D. L. (2002). Report from a symposium on diet and breast cancer. *Cancer Causes Control*, 13, 591–594.
- King, M. C. (1992). Breast cancer genes: How many, where and who are they? *Nature Genetics*, 2, 89–90. Erratum (1992): *Nature Genetics*, 2, 254.
- Klinenberg, E. (2002). *Heat wave: A social autopsy of disaster in Chicago*. Chicago: University of Chicago Press.
- Lancaster, J. M., Wooster, R., Mangion, J., Phelan, C. M., Cochran, C., Gumbs, C., et al. (1996). *BRCA2* mutations in primary breast and ovarian cancer. *Nature Genetics*, 13, 238–240.
- Larsen, P., Kronenberg, H., Melmed, S., & Polonsky, K. (2002). *Williams textbook of endocrinology* (10th ed.). Philadelphia: W. B. Saunders.
- Lee, P. A., Guo, S. S., & Kulin, H. E. (2001). Age of puberty: Data from the United States. *Acta Pathologica, Microbiologica et Immunologica Scandinavica*, 109, 81–88.
- LeFevre, J., & McClintock, M. K. (1988). Reproductive senescence in female rats: A longitudinal study of individual differences in estrous cycles and behavior. *Biology of Reproduction*, 38, 780–789.
- LeFevre, J., & McClintock, M. K. (1991). Isolation accelerates reproductive senescence and alters its predictors in female rats. *Hormones and Behavior*, 25, 258–272.
- Lin, M., Chu, C. C., Lee, H. L., Chang, S. L., Ohasahi, J., Tokunaja, K., et al. (2000). Heterogeneity of Taiwan's indigenous population: Possible relation to prehistoric Mongoloid dispersals. *Tissue Antigens*, 55, 1–9.
- Lindblom, A., Skoog, L., Andersen, T. I., Rotstein, S., Nordenskjold, M., & Larsson, C. (1993). Four separate regions on chromosome 17 show loss of heterozygosity in familial breast carcinomas. *Human Genetics*, 91, 6–12.
- Lund, L. R., Romer, J., Thomasset, N., Solberg, H., Pyke, C., Bissell, M. K., et al. (1996). Two distant phases of apoptosis in mammary gland involution: Proteinase-independent and -independent pathways. *Development*, 122, 181–193.
- Marie Swanson, G., Haslam, S., & Azzouz, F. (2003). Breast cancer among young African-American women: A summary of data and literature and of issues discussed during the Summit Meeting on Breast Cancer Among African American Women, Washington, DC, September 8–10, 2000. *Cancer*, 97, 273–279.
- Martin, L. J., Mahaney, M. C., Bronikowski, A. M., Dee Carey, K., Dyke, B., & Comuzzie, A. G. (2002). Lifespan in captive baboons is heritable. *Mechanisms of Ageing and Development*, 123, 1461–1467.
- Mboya, M. M. (2000). African adolescents and their teachers: Sex and rural-urban comparisons in teachers' perceived behaviors. *Psychological Reports*, 86, 1229–1233.
- McClintock, M. K. (1978). Estrous synchrony in the rat and its mediation by airborne chemical communication (*Rattus norvegicus*). *Hormones and Behavior*, 10, 264–276.
- McClintock, M. K. (1983). Synchronizing ovarian and birth cycles by female pheromones. In D. Müller-Schwarze & R. Silverstein (Eds.), *Chemical signals in vertebrates III* (pp. 158–178). New York: Plenum Press.
- McClintock, M. K. (1984). Estrous synchrony: Modulation of ovarian cycle length by female pheromones. *Physiology and Biology*, 32, 701–705.
- Merajver, S. D., Pham, T. M., Caduf, R. F., Chen, M., Poy, E. L., Cooney, K. A., et al. (1995). Somatic mutations in the *BRCA1* gene in sporadic ovarian tumours. *Nature Genetics*, 9, 439–443.
- Miki, Y., Katagiri, T., Kasumi, F., & Yoshimoto, T. (1996). Mutation analysis in the *BRCA2* gene in primary breast cancers. *Nature Genetics*, 13, 245–247.
- Mikosz, C. A., Brickley, D. R., Sharkey, M. S., Moran, T. W., & Conzen, S. D. (2001). Glucocorticoid receptor-mediated protection from apoptosis is associated with induction of the serine/threonine survival kinase gene, *sgk-1*. *Journal of Biological Chemistry*, 276, 16649–16654.
- Minino, A. M., & Smith, B. L. (2001). *Deaths: Preliminary data for 2000. National Vital Statistics Reports*. Hyattsville: National Center for Health Statistics.
- Mitamura, R., Yano, K., Suzuki, N., Ito, Y., Makita, Y., & Okuna, A. (2000). Diurnal rhythms of luteinizing hormone, follicle-stimulating hormone, testosterone, and estradiol secretion before the onset of female puberty in short children. *Journal of Clinical Endocrinology and Metabolism*, 85, 1074–1080.
- Moran, T. J., Gray, S., Mikosz, C. A., & Conzen, S. D. (2000). The glucocorticoid receptor mediates a survival signal in human mammary epithelial cells. *Cancer Research*, 60, 867–872.
- Morrison, J. A., Barton, B., Biro, F. M., Sprecher, D. L., Falkner, F., & Obarzanek, E. (1994). Sexual maturation and obesity in 9- and 10-year-old black and white girls: The National Heart, Lung, and Blood Institute Growth and Health Study. *Journal of Pediatrics*, 124, 889–895.
- Mul, D., Fredriks, A. M., van Buren, S., Oostdijk, W., Verloove-Vanhorick, S. P., & Wit, J. L. (2001). Pubertal development in the Netherlands, 1965–1997. *Pediatric Research*, 50, 479–486.
- Naidu, A. G., Thomson, S. R., & Nirmul, D. (1989). Giant fibro-adenomas in black and Indian adolescents. *South African Journal of Surgery*, 27, 171–172.
- Neville, M. C., McFadden, T. B., & Forsyth, I. (2002). Hormonal regulation of mammary differentiation and milk secretion. *Journal of Mammary Gland Biology and Neoplasia*, 7, 49–66.
- Patterson, O. (1997). *The ordeal of integration: Progress and resentment in America's "racial" crisis*. Washington, DC: Publishers Group West.
- Phillips, K., Nichol, K., Ozcelik, H., Knight, J., Done, S., Goodwin, P., et al. (1999). Frequency of p53 mutations in breast carcinomas from

- Ashkenazi Jewish carriers of *BRCA1* mutations. *Journal of the National Cancer Institute*, 91, 469–473.
- Rao, V. N., Shao, N., Ahmad, M., & Reddy, E. S. (1996). Antisense RNA to the putative tumor suppressor gene *BRCA1* transforms mouse fibroblasts. *Oncogene*, 12, 523–528.
- Rebeck, T. R., Jaffee, J. M., Walker, A. H., Wein, A. J., & Malkowicz, S. B. (1998). Modification of clinical presentation of prostate tumors by a novel genetic variant in *CYP3A4*. *Journal of the National Cancer Institute*, 90, 1225–1229.
- Reichardt, H. M., Horsch, K., Gröne, H.-J., Kolbus, A., Beug, H., Hynes, N., et al. (2001). Mammary gland development and lactation are controlled by different glucocorticoid receptor activities. *European Journal of Endocrinology*, 145, 519–527.
- Roest Crollius, H., Jaillon, O., Bernot, A., Dasilva, C., Bouneau, L., Fischer, C., et al. (2000). Estimate of human gene number provided by genome-wide analysis using *Tetraodon nigroviridis* DNA sequence. *Nature Genetics*, 25, 235–238.
- Salih, A. K., & Fentiman, I. S. (2001). Breast cancer prevention: Present and future. *Cancer Treatment Reviews*, 27, 261–273.
- Sampson, R. J., Raudenbush, S. W., & Earls, F. (1997). Neighborhoods and violent crime: A multilevel study of collective efficacy. *Science*, 277, 918–924.
- Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1986). The adrenocortical axis in the aged rat: Impaired sensitivity to both fast and delayed feedback inhibition. *Neurobiology of Aging*, 7, 331–335.
- Schmitt, C. A., & Lowe, S. W. (1999). Apoptosis and therapy. *Journal of Pathology*, 187, 127–137.
- Schmitt, F., & Reis-Filho, J. (2003). *c-myc*, not *her-2/neu*, can predict the prognosis of breast cancer patients: How novel, how accurate, and how significant? *Breast Cancer Research*, 5, 188–191.
- Schneider, B., Laubenberger, J., Kommoss, F., Madjar, H., Grone, K., & Langer, M. (1997). Multiple giant fibroadenomas: Clinical presentation and radiologic findings. *Gynecologic and Obstetric Investigation*, 43, 278–280.
- Shiao, Y. H., Chen, V. W., Lehmann, H. P., Wu, X. C., & Correa, P. (1997). Patterns of DNA ploidy and S-phase fraction associated with breast cancer survival in blacks and whites. *Clinical Cancer Research*, 3, 587–592.
- Skolnick, M. H., & Cannon-Albright, L. A. (1992). Genetic predisposition to breast cancer. *Cancer*, 70(suppl.), 1747–1753.
- Skytthe, A., Pedersen, N., Kaprio, J., Stazi, M., Hjelmborg, J., Iachine, I., et al. (2003). Longevity studies in GenomEUtwin. *Twin Research*, 6, 448–454.
- Soung, Y., Lee, J., Kim, S., Park, W., Nam, S., Lee, J., et al. (2004). Somatic mutations of *CASP3* gene in human cancers. *Human Genetics*, 115, 112–115.
- Steele, C., & Sherman, D. A. (1999). The psychological predicament of women on welfare. In D. A. Prentice & D. T. Miller (Eds.), *Cultural divides: Understanding and overcoming group conflict* (pp. 393–428). New York: Russell Sage Foundation.
- Stern, K., & McClintock, M. K. (1998). Regulation of ovulation by human pheromones. *Nature*, 392, 177–179.
- Sun, S., Li, L., & Sun, D. (1999). In situ observation of apoptosis and proliferation in breast cancer and its precancerous lesions. *Zhonghua Zhong Liu Za Zhi*, 21, 447–449.
- Takahashi, H., Chiu, H. C., Bandera, C. A., Behbakht, K., Liu, P. C., Couch, F. J., et al. (1996). Mutations of the *BRCA2* gene in ovarian carcinomas. *Cancer Research*, 56, 2738–2741.
- Teng, D., Bogden, R., Mitchell, J., Baumgard, M., Bell, R., Berry, S., et al. (1996). Low incidence of *BRCA2* mutations in breast carcinoma and other cancers. *Nature Cancer*, 13, 241–244.
- Thompson, T. E., Jensen, R. A., Obermiller, P. S., Page, D. H., & Holt, J. T. (1995). Decreased expression of *BRCA1* accelerates growth and is often present during sporadic breast cancer progression. *Nature Genetics*, 9, 444–450.
- Thyaga Rajan, S., & Felten, D. L. (2002). Modulation of neuroendocrine-immune signaling by L-deprenyl and L-desmethyldeprenyl in aging and mammary cancer. *Mechanisms of Ageing and Development*, 123, 1065–1079.
- van't Veer, L. J., Dai, H., van de Vijver, M. J., He, Y. D., et al. (2002). Gene expression profiling predicts clinical outcome of breast cancer. *Nature*, 415, 530–536.
- Wagner, B. L., Pollio, G., Giangrande, P., Webster, J. C., Breslin, M., Maais, D. E., et al. (1999). The novel progesterone receptor antagonists RTI 3021-012 and RTI 3021-022 exhibit complex glucocorticoid receptor antagonist activities: Implications for the development of dissociated antiprogestins. *Endocrinology*, 140, 1449–1458.
- Waite, L. J. (2000). *The case for marriage: Why married people are happier, healthier, and better off financially (with Maggie Gallagher)*. New York: Doubleday.
- Webster, M., Goya, L., Ge, Y., Maiyar, A., & Firestone, G. (1993). Characterization of *sgk*, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. *Molecular Cell Biology*, 13, 2031–2040.
- Wennink, J. M., Delemarre-van de Waal, H. A., Schoemaker, R., Blaauw, G., & van den Braken, C. (1991). Growth hormone secretion patterns in relation to LH and estradiol secretion throughout normal female puberty. *Acta Endocrinologica*, 124, 129–135.
- Wilkinson, R. G. (1999). *The society and population health reader: Income, inequality and health*. New York: New Press.
- Williams, D. R., Neighbors, H. W., & Jackson, J. S. (2003). Racial/ethnic discrimination and health: Findings from community studies. *American Journal of Public Health*, 93, 200–208.
- Wilson, W. J. (1996). *When work disappears: The world of the new urban poor*. New York: Knopf.
- Wu, T., Mendola, P., & Buck, G. (2002). Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: The Third National Health and Nutrition Examination Survey, 1988–1994. *Pediatrics*, 110, 752–757.
- Wu, W., Chaudhuri, S., Brickley, D. R., Pang, D., Karrison, T., & Conzen, S. D. (2004). Microarray analysis reveals glucocorticoid-regulated survival genes that are associated with inhibition of apoptosis in breast epithelial cells. *Cancer Research*, 64, 1757–1764.
- Yu, J., Hu, S., Wang, J., Wong, G. K., Li, S., Liu, B., et al. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. indica). *Science*, 296, 79–92.
- Zeigler-Johnson, C., Walker, A., Mancke, B., Spangler, E., Jalloh, M., McBride, S., et al. (2002). Ethnic differences in the frequency of prostate cancer susceptibility alleles at *SRD5A2* and *CYP3A4*. *Human Heredity*, 54, 13–21.
- Zeitlin, M., Megawangi, R., Kramer, E., Colletta, N., Babatunde, E., & Garnabm, D. (1995). *Strengthening the family—Implications for international development*. Tokyo: United Nations University Press.