

Perspective

Isolating the Effects of Social Interactions on Cancer Biology

Perspective on Williams et al., p. 850

Brian C. Trainor,^{1,4} Colleen Sweeney⁴ and Robert Cardiff^{2,3}

Abstract This perspective on Williams et al. (beginning on p. 850 in this issue of the journal) examines the connections between biological responses activated during psychosocial stress and mammary tumorigenesis. Experiments in mouse models of cancer are identifying aspects of tumor biology that may be regulated by hormones such as glucocorticoids released during psychosocial stress. Our growing understanding of the actions of glucocorticoids on breast tumors could lead to important changes in cancer treatment.

Psychosocial factors have long been suspected to have important effects on human health (1). Epidemiologic studies of the relationships between breast cancer and psychosocial stress, however, have produced maddeningly inconsistent results. Some studies indicate that depression and lack of social support may be risk factors for breast cancer development and progression (2, 3), whereas others report no link between social stress and breast cancer incidence (4). A weakness common to all of these studies is in not having measured individual physiologic responses to stress. The hypothalamic-pituitary-adrenal (HPA) axis, which stimulates the release of glucocorticoids and epinephrine, is an important stress-activated system. The HPA axis has become a focal point for understanding the effects of stress on health because of its ability to affect a wide range of physiologic processes, including immune function (5). In a natural context, activation of the HPA axis prepares an individual to “fight or flee” from a challenging situation (6). The HPA axis, however, could be chronically activated in the absence of a natural challenge, and it has been proposed that many of the deleterious effects of stress on human health may result from this chronic activation (7). Psychosocial stress in the forms of anxiety, depression, and interpersonal conflict could activate the HPA axis, causing the release of glucocorticoids and epinephrine.

It is becoming increasingly clear that individuals vary in their physiologic responses to stressful situations (8). Therefore, stressful life events are not universally translated into physiologic signals that could affect cancer biology. This may partially explain why epidemiologic studies examining the association between stress and breast cancer have pro-

duced such heterogeneous results. Two general approaches could help resolve the question of whether psychosocial stress indeed affects physiologic processes regulating the development and/or progression of cancer. First, epidemiologic studies need to characterize individual variation in how stressful life events translate into biological signals such as glucocorticoids. Although individual differences in stress hormone responses are not normally collected within large-scale epidemiologic studies, it will be difficult for the field to move forward without this critical information. Second, mechanistic studies can test whether biological signals (such as glucocorticoids) activated during stress regulate cancer biology.

The Conzen group is a leader in the study of how glucocorticoids affect tumors. For example, this group showed that the powerful synthetic glucocorticoid dexamethasone inhibits chemotherapy-induced apoptosis *in vitro* (9). They also recently showed that dexamethasone increased the expression of genes known to inhibit apoptosis in patients with ovarian cancer (10). In addition to suggesting that HPA activation might influence tumor biology, these results are worrying because dexamethasone is routinely administered as an antiemetic during chemotherapy.

As reported in this issue of the journal (11), the Conzen group (Williams et al.) now has used an integrative approach in testing the connections between behavior and cancer, examining the effects of social isolation on mammary tumor growth and gene expression in the FVB-Tg(C3[1]/SV40 T-antigen) mouse model. Using a multidisciplinary approach, these investigators attempted to discover the physiologic and molecular connections between psychological stress and cancer. Although there has been much speculation that HPA activation can affect cancer biology, very few studies have established a link with specific molecular mechanisms regulating tumor initiation or progression. Identifying these molecular mechanisms could lead to new therapeutic targets. Although the authors' innovative approach provided potentially important insights into the connections between social isolation and molecular mechanisms of carcinogenesis, many related dots remain to be connected in this model. A major advantage of mouse models lies in the power to manipulate the environment, gene expression, and physiologic systems. It seems

Authors' Affiliations: Departments of ¹Psychology and ²Pathology, ³Center for Comparative Medicine, University of California Davis, Davis, California; and ⁴University of California Davis Cancer Center, Sacramento, California
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Requests for reprints: Robert Cardiff, Department of Pathology and Laboratory Medicine, School of Medicine, 2795 Second Street, University of California Davis, Davis, CA 95616. Phone: 530-757-3279; Fax: 530-757-3277; E-mail: rdcardiff@ucdavis.edu.

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likely that studies implementing the approach used by Williams et al. will go a long way towards resolving whether psychosocial stress has an important effect on human breast cancers.

Some previous studies have examined the relationship between psychosocial stress and metastatic cancers in rodent models. The vast majority of these studies have used restraint, often considered a "gold standard" in stress biology research. In rodents, restraint produces repeatable, well-characterized increases in glucocorticoid and epinephrine release that mimic stress-induced neuroendocrine responses in humans. One recent study showed that restraint-related stress increased the expression of vascular endothelial growth factor and facilitated vascularization growth of human ovarian carcinoma cells inoculated into nude mice (12). Furthermore, inhibition of the β 2-adrenergic receptor blocked these processes and slowed tumor growth.

Although restraint-related stress has proved to be an important experimental model, its ecologic significance is unclear. For example, how does the experience of restraint in a mouse translate to the human condition? In contrast, it seems intuitive that social isolation in mice is related to the human condition, as social isolation is a risk factor for mortality from cancer and cardiovascular disease (13). Most strains of mice, particularly widely used FVB mice, are highly social and actively seek out social interactions with other mice (14). The effects of social isolation on physiology are less well defined compared with those of restraint-related stress. It seems, however, that isolation-related stress affects HPA function (15). Based on the present results of Williams et al. (11), it also seems that social isolation could have important effects on signaling pathways influencing tumor biology.

Williams et al. found that more and larger tumors developed in socially isolated FVB-Tg(C3[1]/SV40 T-antigen) mice than in group-housed mice. Although the SV40 virus itself is not believed to be involved in human breast cancers, the T-antigens have provided important insights into mammary gland differentiation and tumorigenesis (16). The large T-antigen binds and functionally inactivates the p53 and pRb tumor suppressor proteins (17), and creates an expression profile shared by some aggressive human breast cancers (18). This makes the T-antigen a useful tool for studying mammary tumorigenesis. The authors report that social isolation did not increase C3(1) transgene expression and argue that this observation supports the hypothesis that the C3(1) promoter is not influenced by glucocorticoids. Further investigation of this matter is warranted, however, because this study did not directly examine the effects of glucocorticoids on transgene expression, and androgens have been shown to regulate the activation of the C3(1) promoter (19). Although not well documented in the mammary gland, many other SV40 T-antigen mouse models express synaptophysin (20), a marker of neuroendocrine tumors that are sensitive to circulating hormones (21) such as glucocorticoids. Therefore, glucocorticoids could be interacting with cells expressing the SV40 T-antigen.

Based on their previous work linking dexamethasone and apoptosis, Williams et al. hypothesized that socially isolated mice would have increased glucocorticoids that in turn would reduce apoptosis and increase proliferation in the tissue of mammary intraepithelial neoplasia. They did not find, how-

ever, that social isolation affected apoptosis. SV40 T-antigen-driven carcinomas develop quite rapidly, and this rapidity may have limited the ability to detect an apoptosis effect. The authors did detect the effects of housing conditions on tumor size and onset. They showed that levels of corticosterone (the predominant glucocorticoid in mice) were not different between group-housed and isolated mice prior to mild, short-term restraint. Following this restraint, however, corticosterone secretion was elevated in isolated mice compared with group-housed mice. The effect of social isolation was only detected during mild restraint, suggesting that socially isolated mice may experience exaggerated corticosterone responses to relatively minor everyday stressors (such as cage changing). These increased corticosterone responses in socially isolated mice could have a cumulative effect on apoptosis or cell proliferation, even though such changes were not detected in the present study. The absence of differences in apoptosis could be due to either the relatively fast onset of SV40 T-antigen tumors or other mechanisms. For example, β -adrenergic receptors activated during stress are known to promote angiogenesis in an orthotopic mouse model of ovarian carcinoma (12). There is also evidence that glucocorticoids can promote autophagy in some cell types (22). Although autophagy is a form of cell death, it seems that autophagy can promote the survival of cancer cells in some cases (23). Future studies will be needed to determine whether these mechanisms are affected by social isolation.

The authors used microarray analyses to identify new pathways that might connect social isolation to the increased tumor number and size they observed. They examined gene expression in the mammary glands of mice 15 and 20 weeks of age. More genes were down-regulated in socially isolated versus group-housed mice aged 15 weeks. Of the up-regulated genes in isolated mice, genes related to metabolism, including three genes that are up-regulated in metastatic breast cancer cell lines, received the focus of attention. Using quantitative real-time PCR, the authors showed that acetyl-CoA carboxylase- α (*Acaca*), ATP citrate lyase (*Acly*), and hexokinase 2 (*Hk2*) were up-regulated in mammary glands from isolated mice versus from group-housed mice. Although intriguing, these results are difficult to interpret mechanistically without knowing how social isolation affects these genes' protein levels or how their expression is regulated by stress-related hormones in this mouse model. Future study also will be needed to determine whether changes in gene expression occur in the epithelium, adipocytes, or stromal cells.

The authors make a strong case, however, that these genes could have an important role in influencing the effects of psychosocial stress on mammary tumorigenesis. They note that both *Acly* and *Acaca* are overexpressed in breast carcinoma and that experimental knockdown of these genes inhibits the survival and proliferation of cancer cell lines. In addition, these genes are involved in fatty acid synthesis, which is up-regulated in many types of cancer (24). In particular, expression of fatty acid synthase is associated with a poor prognosis and resistance to therapeutic drugs (25). In the fatty acid synthase pathway, *Acly* produces citrate which is used by acetyl-CoA to produce malonyl-CoA. Malonyl-CoA is a substrate used by fatty acid synthase to produce palmitate and, eventually, fatty acids. The authors note that knockdown of *Acly* expression suppresses lung adenocarcinoma cell growth. Although some questions

of exactly how social isolation affects the expression of *Acly* and *Acaca* remain unanswered, the present results of Williams et al. suggest that future studies of fatty-acid synthesis in mice will need to seriously consider how housing conditions might influence this pathway.

Further study is needed to determine the relative importance of the different components of the HPA axis. Although corticosterone is a prime candidate mechanism linking experience to cancer biology, previous studies have shown that β -adrenergic receptors play an important role in mediating the effects of stress on ovarian carcinoma (12). It also would be interesting to quantify the effects of routine animal husbandry on the secretion of corticosterone and epinephrine and to assess whether these responses are regulated by the social environment. A more complete understanding of how these hormones are secreted under different contexts could provide important insights into how social isolation causes changes in tumor growth. This information could also provide an interesting comparison for human studies; for example, comparing physiologic responses to more major stressful life events (e.g., divorce and foreclosure) with those elicited by lower-grade, daily-life hassles (e.g., commuting and overscheduling). By conducting parallel studies in rodents and humans, it might be possible to correlate behavioral and physiologic responses to different types of stressful events with specific changes in tumor biology (e.g., apoptosis, angiogenesis, and metastasis).

Based on the variable relevant epidemiologic data (2–4), skeptics might argue that results linking behavioral manipulations such as social isolation to changes in tumor biology in a mouse have limited relevance to human health. Too much reliance on the epidemiologic data could be a mistake, however, because they do not incorporate individual differences in physiologic stress responses that could introduce variances in epidemiologic results. For example, recent studies showed that “resilient” individuals (those who adapt successfully to acute stress) exhibit different neurobiological, hormonal, and behavioral traits in the face of acute stress

compared with nonresilient individuals (8). Resilient individuals are more likely to deal with stressful life events with proactive strategies such as problem solving than with reactive strategies of avoidance or denial. More relevant to health outcomes, resilient individuals are more likely to efficiently terminate stress-induced HPA activation, thereby limiting exposure to high levels of glucocorticoids (26). Proactive and reactive (passive) coping strategies have been identified in rodents, with reactive individuals showing increased avoidance behavior or immobility in stressful situations (27). In rats, it seems that proactive coping strategies reduce long-term activation of the HPA axis, which could have important health implications. Female rats are known to exhibit proactive and reactive strategies. Compared with proactive females, reactive females showed reduced exploratory behavior and increased corticosterone secretion when exposed to a large, complex novel cage (containing large objects, tunnels, et cetera). Reactive rats had shorter life spans and were more likely to develop mammary tumors versus proactive rats (28). These findings are similar to individual variations in coping strategies reported for highly inbred mouse lines such as C57Bl/6 (29).

Individual differences in stress-induced behavioral responses of both humans and animals are observed under highly controlled laboratory environments, even in studies of highly inbred mice. This suggests that detecting links between stress hormones and cancer biology within the genetic and environmental heterogeneity of human populations will be a substantial challenge. The article by the Conzen group is an important step towards establishing the connections between behavioral interactions and molecular oncology, offering insight and alternate hypotheses that should stimulate more mechanistic research in this important field.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Dunn AJ. Psychoneuroimmunology: introduction and general perspectives. In: Leonard B, Miller K, editors. *Stress, the immune system and psychiatry*. New York: John Wiley & Sons; 1995. p. 1–16.
- Spiegel D, Giese-Davis J. Depression and cancer: mechanisms and disease progression. *Biol Psychiatry* 2003;54:269–82.
- Lillberg K, Verkasalo PK, Kaprio J, Teppo L, Helenius H, Koskenvuo M. Stressful life events and risk of breast cancer in 10,808 women: a cohort study. *Am J Epidemiol* 2003;157:415–23.
- Surtees PG, Wainwright NW, Luben RN, Khaw KT, Bingham SA. No evidence that social stress is associated with breast cancer incidence. *Breast Cancer Res Treat*. In press 2009.
- Nelson RJ, Demas GE, Klein SL, Kriegsfeld LJ. Seasonal patterns of stress, immune function, and disease. New York: Cambridge University Press; 2002.
- McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev* 2007;87:873–904.
- Sapolsky RM. Why zebras don't get ulcers. New York: Henry Holt; 2004.
- Feder A, Nestler EJ, Charney DS. Psychobiology and molecular genetics of resilience. *Nat Rev Neurosci* 2009;10:446–57.
- Wu W, Chaudhuri S, Brickley DR, Pang D, Karrison T, Conzen SD. Microarray analysis reveals glucocorticoid-regulated survival genes that are associated with inhibition of apoptosis in breast epithelial cells. *Cancer Res* 2004;64:1757–64.
- Melhem A, Yamada SD, Fleming GF, et al. Administration of glucocorticoids to ovarian cancer patients is associated with expression of the anti-apoptotic genes SGK1 and MKP1/DUSP1 in ovarian tissues. *Clin Cancer Res* 2009;15:3196–204.
- Williams B, Pang D, Delgado B, et al. Chronic social isolation is associated with altered gene expression and increased tumor growth in transgenic mice. *Cancer Prev Res*. In press 2009.
- Thaker PH, Han LY, Kamat AA, et al. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat Med* 2006;12:939–44.
- Hawkey LC, Cacioppo JT. Loneliness and pathways to disease. *Brain Behav Immun* 2003;17:S98–105.
- Brodtkin ES, Hagemann A, Nemetski SM, Silver LM. Social approach-avoidance behavior of inbred mouse strains toward DBA/2 mice. *Brain Res* 2004;1002:151–7.
- Grippe AJ, Gerena D, Huang J, et al. Social isolation induces behavioral and neuroendocrine disturbances relevant to depression in female and male prairie voles. *Psychoneuroendocrinology* 2007;32:966–80.
- Goetz F, Tzeng YJ, Guhl E, Merker J, Graessmann M, Graessmann A. The SV40 small t-antigen prevents mammary gland differentiation and induces breast cancer formation in transgenic mice; truncated large T-antigen molecules harboring the intact p53 and pRb binding region do not have this effect. *Oncogene* 2001;20:2325–32.
- May P, May E. Twenty years of p53 research: structural and functional aspects of the p53 protein. *Oncogene* 1999;18:7621–36.
- Deeb KK, Michalowska AM, Yoon C-y, et al. Identification of an integrated SV40 T/t-antigen cancer signature in aggressive human breast, prostate, and lung carcinomas with poor prognosis. *Cancer Res* 2007;67:8065–80.

19. Shibata MA, Jorcyk CL, Devor DE, et al. Altered expression of transforming growth factor β s during urethral and bulbourethral gland tumor progression in transgenic mice carrying the androgen-responsive C3(1) 5' flanking region fused to SV40 large T antigen. *Carcinogenesis* 1998;19:195–205.
20. Couto SS, Cardiff RD. The genomic revolution and endocrine pathology. *Endocr Pathol* 2008;19:139–47.
21. Gould VE, Wiedenmann B, Lee I, et al. Synaptophysin expression in neuroendocrine neoplasms as determined by immunocytochemistry. *Am J Pathol* 1987;126:243–57.
22. Laane E, Tamm KP, Buentke E, et al. Cell death induced by dexamethasone in lymphoid leukemia is mediated through initiation of autophagy. *Cell Death Differ* 2009;16:1018–29.
23. Brech A, Ahlquist T, Lothe RA, Stenmark H. Autophagy in tumour suppression and promotion. *Mol Oncol* 2009;3:366–375.
24. Kuhajda FP. Fatty acid synthase and cancer: new application of an old pathway. *Cancer Res* 2006;66:5997–80.
25. Liu H, Liu Y, Zhang J-T. A new mechanism of drug resistance in breast cancer cells: fatty acid synthase overexpression-mediated palmitate overproduction. *Mol Cancer Ther* 2008;7:263–70.
26. de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 2005;6:463–75.
27. Koolhaas JM, De Boer SF, Buwalda B, van Reenen K. Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. *Brain Behav Evol* 2007;70:218–26.
28. Cavigelli SA, Yee JR, McClintock MK. Infant temperament predicts life span in female rats that develop spontaneous tumors. *Horm Behav* 2006;50:454–62.
29. Krishnan V, Han M-H, Graham DL, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell* 2007;131:391–404.

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