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Individual differences in pre-carcinogen cytokine and corticosterone concentrations and depressive-like behavior predict tumor onset in rats exposed to a carcinogen

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Summary

Individual variation in the susceptibility to chronic disease can be attributed to both genetic and environmental factors. Measures of the immune, nervous, and endocrine systems are predictive of survival outcomes after a chronic disease is diagnosed. However, determining biomarkers or “traits” that predict risk before chronic disease development remains elusive. In this study, natural individual variation in circulating cytokines, corticosterone, and depressive-like behaviors (using the Porsolt forced swim test) were measured in female rats before induction of mammary tumors using a chemical carcinogen (N-nitroso-N-methylurea). Early tumor onset was associated with relatively high (but within the physiologically typical range) circulating cytokine concentrations (IL-1 α , IL-1 β , TNF α) and depressive-like behavior and with relatively low corticosterone concentrations, all of which were assessed at baseline before carcinogen treatment. Multiple regression analyses indicated that IL-1 β was primarily responsible for the variation in tumor onset when controlling for corticosterone concentration. These results suggest that the susceptibility to tumor initiation and/or growth may be related to individual differences in baseline immune and endocrine physiology and emotional tone present at the time of carcinogen exposure. Investigation of the mechanistic relevance of these individual differences may lead to prophylactic approaches to cancer treatment in the context of carcinogen exposure.

Keywords

Tumor onset; Individual differences; Biomarkers; Depression; Biobehavioral; Cancer susceptibility; Cancer risk

1. Introduction

Individual variation in the susceptibility to chronic diseases can be attributed to both genetic and environmental factors. The influence of these factors on inflammation and hypothalamic-pituitary-adrenal (HPA) axis responses have been consistently linked to

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Conflict of interest

The authors report no conflicts of interest.

depression (Dantzer et al., 2008; Leonard and Maes, 2012). In turn, interactions among these three biological systems (i.e., immune, endocrine, nervous) also influence the progression and severity of peripheral disease (Kiecolt-Glaser et al., 2002; Dantzer et al., 2008). For example, in cancer patients, relationships among symptoms of depression, circulating inflammatory cytokine concentrations, and HPA axis activity can predict survival rates, recurrence rates, behavioral comorbidities, and treatment outcomes (Allen-Mersh et al., 1998; Dunlop and Campbell, 2000; Cohen et al., 2002; Capuron et al., 2004; Costanzo et al., 2005; Antoni et al., 2006). These factors may collectively contribute to cancer risk or onset (Lutgendorf, 2003; Segerstrom, 2003) earlier on, but such a hypothesis is difficult to address conclusively in patients (Park and Kang, 2006; Whalley et al., 2007; but see van den Biggelaar et al., 2007). Understanding the role of individual differences in physiological or behavioral traits on cancer susceptibility is particularly relevant for cancers for which no singular cause has been identified (e.g., breast cancer; Stevens, 2009).

Cytokine production is both a cause and a consequence of cancers. Cytokines control inflammation via their production and release by immune cells; and their actions are negatively regulated by elevated glucocorticoids (De Bosscher and Haegeman, 2009). Pre-existing chronic inflammation, even at subpyrogenic levels, is hypothesized to be a causal factor in some cancers (Peek et al., 2005; Prendergast et al., 2010; Grivennikov and Karin, 2011). Cytokines are also produced by tumor cells and are involved in all stages of tumor progression from initiation to metastasis (Coussens and Werb, 2002; Yoo et al., 2009). The HPA axis regulates cytokine-mediated inflammation, in part, via glucocorticoids, which inhibit cytokine production and signaling. Psychological stressors (e.g., social isolation), which also trigger glucocorticoid release, exacerbate tumor growth in cancer models (Hermes et al., 2009; Williams et al., 2009). Accordingly, a psychological depressive-like state may alter disease susceptibility or severity (O'Neil and Moore, 2003).

Individual differences in physiological and behavioral variables can predict some immune responses and chronic disease outcomes. For example, Wistar rats low in exploratory behavior also exhibit low corticosterone responses to novelty and high Th1/Th2 cytokine ratios (proinflammatory) to an immune challenge. In addition, they are more susceptible to experimental autoimmune encephalomyelitis (EAE) and grow larger implanted tumors (Cools et al., 1993; Kavelaars et al., 1997; Teunis et al., 2002). In another neophobic rat model, blunted corticosterone responses earlier in life predict spontaneous mammary tumors (Cavigelli et al., 2006). Finally, mice that are high in anxiety-like behavior using an elevated plus maze exhibit a greater skin tumor burden than those with low anxiety-like behavior (Dhabhar et al., 2012). Although these studies provide categorical links between biobehavioral measures, immune function, and disease progression, the quantitative nature of such relations has not been addressed prospectively.

This experiment tested the hypothesis that individual differences in cytokine production, HPA activity, and emotional behavior predict mammary tumor onset following chemical carcinogen exposure in female rats. In a companion experiment, depressive-like behaviors were measured in rats prior to tumor-induction and tumor onset was recorded. Evidence suggesting a predictive role of these biobehavioral markers on later cancer onset would

provide novel quantitative insights into the mechanism by which individual differences in behavior and physiology modulate disease susceptibility.

2. Methods

2.1. Animals

Nulliparous, female Wistar rats (Harlan, Indianapolis, IN, USA) were used in these experiments ($n = 37$). Female siblings from 5 different dams were weaned at 19–24 days of age and group-housed (2–3 per cage) in polypropylene cages (25.9 cm × 47.6 cm × 20.9 cm high) with a constant temperature and humidity of 22 ± 1 °C and $50 \pm 5\%$, respectively, and *ad libitum* access to food (Teklad 2918 rodent diet) and filtered tap water. Rats were housed under a 16 h light/day light–dark cycle (lights off at 16:00 h CST). All experiments conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Chicago. All efforts were made to minimize animal suffering and to reduce the number of rats used.

3. Experiment 1: Predictive value of individual differences in cytokines and corticosterone on tumor onset

3.1. Pre-carcinogen cytokine and corticosterone concentrations

Nineteen naïve rats were used in this experiment. On Day 0, resting blood was sampled retro-orbitally from all rats (30–37 days of age) before carcinogen injection (as described below) for cytokine and corticosterone assessments. Under 3% isoflurane anesthesia, approximately 200 µl of blood was obtained from the retro-orbital sinus using heparinized capillary tubes within 2 min of handling. All blood samples were collected on the same day within a 2 h period approximately 4 h before lights-off. Two days later, rats received carcinogen treatments and tumor onset was recorded as described below.

4. Experiment 2: Predictive value of individual differences in depressive-like behavior on tumor onset

4.1. Depressive-like behavior prior to tumor induction: Porsolt forced swim test

A separate cohort of 18 rats was tested using a standard measure of depressive-like behavior. The first day of testing (pre-test) consisted of a 15-min exposure to this paradigm. The next day, rats were exposed to the same paradigm for a 5-min (testing) period. The time spent swimming and climbing (escape behaviors) and floating (behavioral despair) was estimated using a real-time sampling technique (Detke et al., 1995). In this test, behavioral despair is operationally defined as the time spent floating (versus active escape behaviors) in an inescapable water tank (27 °C, 40 cm deep × 30 cm diameter). Floating behavior in this paradigm is reversible by antidepressant treatments (Porsolt et al., 1977). Two days after behavioral testing, rats were treated with carcinogen (as described below).

4.2. Tumor induction

Two days after blood sampling or behavioral testing, all rats received an injection (IP, 50 mg/kg), of a standard chemical carcinogen (N-nitroso-N-methylurea [NMU] in saline [pH

4.0]; Sigma, St. Louis, MO, USA) (Thompson and Adlakha, 1991; Thompson et al., 1992). NMU induces >90% malignant mammary tumors (ductal carcinomas) in rats that are comparable in origin and mutation to this most common type of breast tumor in women (Zarbl et al., 1985; Thompson et al., 1995; Chan et al., 2005). Body mass was measured weekly and weekly tumor palpation commenced 4 weeks post-injection (onset of palpable [>3 mm] tumors range: 5–11 weeks post-NMU injection).

4.3. Cytokine analysis

IL-1 α , IL-1 β , and TNF α concentrations were measured simultaneously in duplicate in plasma samples (12 μ l) using multiplex fluorescent bead assays (Bio-Plex Cytokine Panel; Bio-Rad, Hercules, CA, USA) validated for use in this species (Hulse et al., 2004). Sample preparation and assay procedures were performed according the manufacturer's protocol at the Flow Cytometry Facility at University of Chicago (Pyter et al., 2009). Samples from 3 randomly chosen rats were not included in this assay due to space limitations. The mean intra-assay CV was 10% and the mean inter-assay ($n = 2$ plates) CV was 6%. The lowest level of detection was 1.95 pg/ml for IL-1 α and IL-1 β and 7.81 pg/ml for TNF α .

4.4. Corticosterone measurement

Corticosterone was measured in duplicate samples using EIAs (Correlate-EIA Kit, Assay Designs, Ann Arbor, MI, USA) according to the manufacturer's instructions in plasma samples diluted 1:60. The corticosterone EIA had a sensitivity of <27.0 pg/ml, an intra-assay CV of 8%, and an inter-assay CV of 10%.

4.5. Statistical analyses

StatView 5.0 software (v. 5.0.1, SAS, Cary, NC, USA) was used to perform all statistics. Simultaneous multiple linear regression analyses were used to predict tumor onset. First, individual cytokines or resting corticosterone was entered into the model as independent variables. Second, given the high number of predictors for the modest samples size, a simultaneous multiple regression model with all the cytokines and corticosterone together was not used. Instead, corticosterone was included in addition to each individual cytokine in separate multiple regression models. Evaluation of differences between median split early and late onset tumor groups was performed using Student's t -tests. Differences were considered significant if $p < 0.05$.

5. Results

5.1. Experiment 1: Pre-carcinogen cytokine and corticosterone concentrations predict tumor onset

Simple and multiple linear regression analyses were conducted to examine the relationship between tumor onset and various predictors, and among the various predictors. Pre-carcinogen concentrations of IL-1 α , IL-1 β , and TNF α were each negatively correlated with the time of tumor onset (Fig. 1A–C). A median split was used to divide tumor onsets into early and late onset groups (median $\pm$ SD: 7.0 $\pm$ 1.9 weeks post-carcinogen treatment; early mean $\pm$ SEM: 5.8 $\pm$ 0.29, $n = 10$; late mean $\pm$ SEM: 9.0 $\pm$ 1.1, $n = 9$). Pre-carcinogen concentrations of IL-1 β were significantly higher in rats that eventually exhibited early

tumor onsets compared with those that exhibited late onsets ($p < 0.05$; Fig. 1B), but this effect was not evident for IL-1 α or TNF α .

Plasma corticosterone concentrations, measured before carcinogen treatment, were significantly and positively correlated with tumor onset (Fig. 2). Pre-treatment body masses did not differ between onset groups (mean $\pm$ SEM, early onset: 177.7 ± 6.3 g; late onset: 188.3 ± 2.5 g; $p > 0.05$; data not shown).

Simple linear regression analyses were used to examine the relations between the predictors of tumor onset: resting pre-carcinogen corticosterone and circulating cytokines. Pre-carcinogen circulating corticosterone was significantly negatively correlated with pre-carcinogen circulating IL-1 β and TNF α , but not IL-1 α (Fig. 3 and Table 1).

When pre-carcinogen corticosterone values were added as another independent variable to multiple regression models with either IL-1 α , IL-1 β , or TNF α , only IL-1 β had a significant negative regression weight (Table 2; $F_{2,14} = 4.1$, $p < 0.05$), indicating that rats with higher baseline IL-1 β had earlier tumor onsets when controlling for effects of corticosterone.

5.2. Experiment 2: Pre-carcinogen depressive-like behaviors predict tumor onset

Floating behavior measured prior to carcinogen treatment was significantly and negatively correlated with the time of tumor onset (Fig. 4). A median split (median $\pm$ SD: 7.5 ± 1.7 weeks post-carcinogen treatment) was used to divide tumor onsets into early (mean $\pm$ SEM: 6.2 ± 0.3 , $n = 9$) and late (mean $\pm$ SEM: 9.1 ± 0.3 , $n = 9$) tumor onsets. Based on this criterion, baseline (pre-carcinogen) floating behavior did not differ between rats that eventually developed early versus late tumors ($p > 0.05$; Fig. 4).

6. Discussion

In a carcinogen-induced mammary tumor model, baseline (pre-carcinogen) circulating pro-inflammatory cytokine concentrations (IL-1 β , IL-1 α , TNF α) were negatively correlated with mammary tumor onset, and baseline corticosterone concentrations were positively correlated with tumor onset. Additionally, pre-carcinogen depressive-like behaviors predicted earlier tumor onset. In a multiple regression analysis that controlled for corticosterone concentrations, only IL-1 β concentrations significantly explained the variance in tumor onset. The results indicate that individual differences in baseline immune and endocrine physiology, and in behavior, predict mammary tumor onset. This mammary tumor model is suitable for further investigation of how natural variation in physiology and behavior affects cancer onset because of the broad range of time over which individual rats develop tumors following carcinogen treatment.

Inflammation has been hypothesized to promote cancer development via oxidative stress effects on cell repair mechanisms (Khansari et al., 2009; Yoo et al., 2009). The present data expand upon these findings by suggesting that basal cytokine concentrations may also play a role in individuals' susceptibility to carcinogen-induced tumor formation. Given that the present snapshot of biological measures was taken in the absence of any overt manipulation in healthy rats, we propose that the data represent a glimpse of natural, individual variation

or an attempt to identify “traits” as opposed to transient “states”. Indeed, the ranges of cytokine concentrations observed in this study are consistent with previously reported baseline values in female rats using this technology (Hulse et al., 2004; IL-1 α : 0–100 pg/ml, IL-1 β : 0–200 pg/ml, TNF α : 0–800 pg/ml). Additionally, attention to rapid blood sampling (>2 min) in this experiment avoided the potential for the isoflurane anesthesia used during collection to interfere with either resting glucocorticoid (Riley, 1981) or cytokine concentrations (Hofstetter et al., 2005). Future studies will examine the hypotheses that these individual cytokines exert their tumor-promoting effects directly, indirectly (Grivennikov and Karin, 2011), or via the creation of an environment permissive for tumor growth.

Lower corticosterone concentrations were associated with early tumor onset and were also inversely correlated with pre-carcinogen proinflammatory cytokines (TNF α and IL-1 β) in this study. This is consistent with the inverse relationship between inflammation and glucocorticoids (Dantzer et al., 1999) and suggests that these circulating factors retain a similar homeostatic balance even in the absence of an experimental homeostatic challenge (e.g., infection, stressor).

The observed relationship between low resting corticosterone concentrations and early cancer onset is consistent with previous research demonstrating that lower HPA axis responses to stressors or lower baseline corticosterone concentrations may predict tumor development (Strange et al., 2000; Cavigelli et al., 2008; Yee et al., 2008). Additionally, rat strains that are characterized by blunted HPA axis responses to stressors, are more susceptible to experimental autoimmune encephalomyelitis (EAE) (Gasser et al., 1973) and arthritis than strains that are not (Sternberg et al., 1989; but see Chover-Gonzalez et al., 2000). Manipulation of glucocorticoids reverses the susceptibility consequences in both of these rat models (Sternberg et al., 1989). Current research focused on how stress influences tumor biology indicates that stress-induced adrenaline and noradrenaline release play an arguably larger role than glucocorticoids in cancer onset and progression (Powe and Entschladen, 2011), although these hormones are not stimulated and therefore unlikely to play a role in the present unprovoked paradigm.

The proinflammatory cytokines investigated here are related and redundant (Yadav and Sarvetnick, 2003); however, when differences in corticosterone values were controlled for in the multiple regression analyses, only IL-1 β significantly predicted variation in tumor onset, suggesting that corticosterone concentrations may be less directly related. This observation compliments previous studies in which genetic polymorphisms of the IL-1 β gene that result in greater IL-1 β protein production are associated with higher risk, severity of disease, and mortality in breast cancer patients (Snoussi et al., 2005; He et al., 2011). While others have shown that the concentration of tumor microenvironment-derived IL-1 β positively correlates with tumor invasiveness and angiogenesis (Voronov et al., 2003; Song et al., 2003), host-derived IL-1 β is also associated with rapid tumorigenesis in studies of IL-1 β knockout mice (Krelin et al., 2007). Therefore, there is potential for systemic, not just local, IL-1 β to modulate cancer susceptibility.

Pre-carcinogen depressive-like behavior significantly predicted when tumors would first appear. Earlier tumor onset in rats that were initially behaviorally phenotyped to have elevated depressive-like behavior is consistent with the hypothesis that emotional tone contributes to cancer risk in humans (Antoni et al., 2006; Armaiz-Pena et al., 2009). To our knowledge, this is the first study to determine that natural variation in standard rodent depressive-like behavior predisposes rats to earlier tumor growth. Prospective investigations of the relations between naïve behavioral profile and subsequent disease development may provide a useful model for how dispositional traits influence disease susceptibility.

Preclinical models of depression typically consist of repeated exposure to stressors during adulthood; this has a consequence of entangling the effects of stress experience from the potential effects of emotional traits on disease outcomes. Thus, models that are characterized by classifying individuals based on natural differences in neophobic or anxiety-like behaviors may come closer to modeling innate differences in emotional disposition. The present results are consistent with studies in which rodents that are selected for reduced exploratory behavior to novelty (apomorphine-unsusceptible Wistar line) or display increased anxiety-like behavior in an elevated plus maze also grow larger tumors and/or more lung metastases (Teunis et al., 2002; Dhabhar et al., 2012). However, in the former study, tumor onset could not be measured and in the latter, potentially related baseline physiological “traits” were not assessed.

Other work has also revealed associations between neophobic behavior and chronic disease which are accompanied by differences in cytokine and glucocorticoid responses. Rats with low exploratory behavior exhibit an elevated inflammatory response to an immune challenge (Kavelaars et al., 1997) and dampened ACTH and glucocorticoid responses to a stressor (Cools et al., 1993; Rots et al., 1996). Relatively high rat neophobic behavior in infancy is also associated with earlier spontaneous mammary tumors and death compared with that of less neophobic siblings (Cavigelli et al., 2006). Blunted corticosterone responses to a stressor are observed in neophobic rats in the absence of coincidentally elevated TNF α responses (Cavigelli et al., 2008). In contrast, elevated HPA axis responses to a stressor coincided with low learned helplessness behavior and subsequently exacerbated adjuvant arthritic inflammation (Chover-Gonzalez et al., 2000). Further studies using the present model are necessary to simultaneously examine the relationship between depressive-like behavior, cytokines, and glucocorticoids within individuals, but may identify a complex relationship among these traits.

In summary, the present data suggest that naturally varying circulating proinflammatory cytokines and glucocorticoid concentrations predict the timing of tumor development. These hormones and cytokines (low corticosterone, high proinflammatory cytokines) may create a permissive physiological environment for tumor development. Depressive-like behavior also predicted the onset of carcinogen-induced mammary tumors, although the independence of depressive-like behavior from corticosterone and cytokines could not be examined in the present study. Although the data do not permit mechanistic or causal insights into these associations, they suggest that the susceptibility to tumor initiation and/or growth may be related to individual differences in physiology and emotional tone present at the time of

carcinogen exposure. Understanding the mechanistic relevance of these individual differences may facilitate prophylactic approaches to cancer treatment.

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References

- Allen-Mersh TG, Glover C, Fordy C, Henderson DC, Davies M. Relation between depression and circulating immune products in patients with advanced colorectal cancer. *J R Soc Med.* 1998; 91:408–413. [PubMed: 9816354]
- Antoni MH, Lutgendorf SK, Cole SW, Dhabhar FS, Sephton SE, McDonald PG, Stefanek M, Sood AK. The influence of bio-behavioural factors on tumour biology: pathways and mechanisms. *Nat Rev Cancer.* 2006; 6:240–248. [PubMed: 16498446]
- Armaiz-Pena GN, Lutgendorf SK, Cole SW, Sood AK. Neuroendocrine modulation of cancer progression. *Brain Behav Immun.* 2009; 23:10–15. [PubMed: 18638541]
- Capuron L, Ravaud A, Miller AH, Dantzer R. Baseline mood and psychosocial characteristics of patients developing depressive symptoms during interleukin-2 and/or interferon-alpha cancer therapy. *Brain Behav Immun.* 2004; 18:205–213. [PubMed: 15050647]
- Cavigelli SA, Bennett JM, Michael KC, Klein LC. Female temperament, tumor development and life span: relation to glucocorticoid and tumor necrosis factor alpha levels in rats. *Brain Behav Immun.* 2008; 22:727–735. [PubMed: 18155400]
- Cavigelli SA, Yee JR, McClintock MK. Infant temperament predicts life span in female rats that develop spontaneous tumors. *Horm Behav.* 2006; 50:454–462. [PubMed: 16836996]
- Chan MM, Lu X, Merchant FM, Iglehart JD, Miron PL. Gene expression profiling of NMU-induced rat mammary tumors: cross species comparison with human breast cancer. *Carcinogenesis.* 2005; 26:1343–1353. [PubMed: 15845649]
- Chover-Gonzalez AJ, Jessop DS, Tejedor-Real P, Gibert-Rahola J, Harbuz MS. Onset and severity of inflammation in rats exposed to the learned helplessness paradigm. *Rheumatology (Oxf).* 2000; 39:764–771.
- Cohen M, Klein E, Kuten A, Fried G, Zinder O, Pollack S. Increased emotional distress in daughters of breast cancer patients is associated with decreased natural cytotoxic activity, elevated levels of stress hormones and decreased secretion of Th1 cytokines. *Int J Cancer.* 2002; 100:347–354. [PubMed: 12115552]
- Cools AR, Rots NY, Ellenbroek B, de Kloet ER. Bimodal shape of individual variation in behavior of Wistar rats: the overall outcome of a fundamentally different make-up and reactivity of the brain, the endocrinological and the immunological system. *Neuropsychobiology.* 1993; 28:100–105. [PubMed: 8255403]
- Costanzo ES, Lutgendorf SK, Sood AK, Anderson B, Sorosky J, Lubaroff DM. Psychosocial factors and interleukin-6 among women with advanced ovarian cancer. *Cancer.* 2005; 104:305–313. [PubMed: 15954082]
- Coussens LM, Werb Z. Inflammation and cancer. *Nature.* 2002; 420:860–867. [PubMed: 12490959]
- Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci.* 2008; 9:46–56. [PubMed: 18073775]
- Dantzer, R.; Wollman, EE.; Yirmiya, R., editors. *Cytokines, Stress, and Depression.* Kluwer Academic/Plenum Publishers; New York: 1999.

- De Bosscher K, Haegeman G. MiniReview: latest perspectives on antiinflammatory actions of glucocorticoids. *Mol Endocrinol*. 2009; 23:281–291. [PubMed: 19095768]
- Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology (Berl)*. 1995; 121:66–72. [PubMed: 8539342]
- Dhabhar FS, Saul AN, Holmes TH, Daugherty C, Neri E, Tillie JM, Kusewitt D, Oberszyn TM. High-anxious individuals show increased chronic stress burden, decreased protective immunity, and increased cancer progression in a mouse model of squamous cell carcinoma. *PLoS One*. 2012; 7:e33069. [PubMed: 22558071]
- Dunlop RJ, Campbell CW. Cytokines and advanced cancer. *J Pain Symptom Manage*. 2000; 20:214–232. [PubMed: 11018340]
- Gasser DL, Newlin CM, Palm J, Gonatas NK. Genetic control of susceptibility to experimental allergic encephalomyelitis in rats. *Science*. 1973; 181:872–873. [PubMed: 4125048]
- Grivennikov SI, Karin M. Inflammatory cytokines in cancer: tumour necrosis factor and interleukin 6 take the stage. *Ann Rheum Dis*. 2011; 70 (Suppl 1):i104–i108. [PubMed: 21339211]
- He B, Zhang Y, Pan Y, Xu Y, Gu L, Chen L, Wang S. Interleukin 1 beta promoter polymorphism and cancer risk: evidence from 47 published studies. *Mutagenesis*. 2011; 26:637–642. [PubMed: 21653279]
- Hermes GL, Delgado B, Tretiakova M, Cavigelli SA, Krausz T, Conzen SD, McClintock MK. Social isolation dysregulates endocrine and behavioral stress while increasing malignant burden of spontaneous mammary tumors. *Proc Natl Acad Sci USA*. 2009; 106:22393–22398. [PubMed: 20018726]
- Hofstetter C, Flondor M, Boost KA, Koehler P, Bosmann M, Pfeilschifter J, Zwissler B, Mühl H. A brief exposure to isoflurane (50 s) significantly impacts on plasma cytokine levels in endotoxemic rats. *Int Immunopharmacol*. 2005; 5:1519–1522. [PubMed: 16023603]
- Hulse RE, Kunkler PE, Fedynyshyn JP, Kraig RP. Optimization of multiplexed bead-based cytokine immunoassays for rat serum and brain tissue. *J Neurosci Methods*. 2004; 136:87–98. [PubMed: 15126049]
- Kavelaars A, Heijnen CJ, Ellenbroek B, van Loveren H, Cools A. Apomorphine-susceptible and apomorphine-unsusceptible Wistar rats differ in their susceptibility to inflammatory and infectious diseases: a study on rats with group-specific differences in structure and reactivity of hypothalamic-pituitary-adrenal axis. *J Neurosci*. 1997; 17:2580–2584. [PubMed: 9065517]
- Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Pat Inflamm Allergy Drug Discov*. 2009; 3:73–80. [PubMed: 19149749]
- Kiecolt-Glaser JK, McGuire L, Robles TF, Glaser R. Psychoneuroimmunology: psychological influences on immune function and health. *J Consult Clin Psychol*. 2002; 70:537–547. [PubMed: 12090368]
- Krelin Y, Voronov E, Dotan S, Elkabets M, Reich E, Fogel M, Huszar M, Iwakura Y, Segal S, Dinarello CA, Apte RN. Interleukin-1beta-driven inflammation promotes the development and invasiveness of chemical carcinogen-induced tumors. *Cancer Res*. 2007; 67:1062–1071. [PubMed: 17283139]
- Leonard B, Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. *Neurosci Biobehav Rev*. 2012; 36:764–785. [PubMed: 22197082]
- Lutgendorf SK. Individual differences and immune function: implications for cancer. *Brain Behav Immun*. 2003; 17 (Suppl 1):S106–S108. [PubMed: 12615194]
- O'Neil MF, Moore NA. Animal models of depression: are there any? *Hum Psychopharmacol*. 2003; 18:239–254. [PubMed: 12766928]
- Park NJ, Kang DH. Breast cancer risk and immune responses in healthy women. *Oncol Nurs Forum*. 2006; 33:1151–1159. [PubMed: 17149398]

- Peek RM Jr, Mohla S, DuBois RN. Inflammation in the genesis and perpetuation of cancer: summary and recommendations from a national cancer institute-sponsored meeting. *Cancer Res.* 2005; 65:8583–8586. [PubMed: 16204020]
- Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature.* 1977; 266:730–732. [PubMed: 559941]
- Powe GD, Entschladen F. Targeted therapies: using beta-blockers to inhibit breast cancer progression. *Nat Rev Clin Oncol.* 2011; 8:511–512. [PubMed: 21808268]
- Prendergast GC, Metz R, Muller AJ. Towards a genetic definition of cancer-associated inflammation: role of the IDO pathway. *Am J Pathol.* 2010; 176:2082–2087. [PubMed: 20228228]
- Pyter LM, Pinos V, Galang JA, McClintock MK, Prendergast BJ. Peripheral tumors induce depressive-like behaviors and cytokine production and alter hypothalamic-pituitary-adrenal axis regulation. *Proc Natl Acad Sci USA.* 2009; 106:9069–9074. [PubMed: 19451634]
- Riley V. Psychoneuroendocrine influences on immunocompetence and neoplasia. *Science.* 1981; 212:1100–1109. [PubMed: 7233204]
- Rots NY, Cools AR, Oitzl MS, de Jong J, Sutanto W, de Kloet ER. Divergent prolactin and pituitary-adrenal activity in rats selectively bred for different dopamine responsiveness. *Endocrinology.* 1996; 137:1678–1686. [PubMed: 8612501]
- Segerstrom SC. Individual differences, immunity, and cancer: lessons from personality psychology. *Brain Behav Immun.* 2003; 17 (Suppl 1):S92–S97. [PubMed: 12615192]
- Snoussi K, Strosberg AD, Bouaouina N, Ben Ahmed S, Chouchane L. Genetic variation in pro-inflammatory cytokines (interleukin-1beta, interleukin-1alpha and interleukin-6) associated with the aggressive forms, survival, and relapse prediction of breast carcinoma. *Eur Cytokine Netw.* 2005; 16:253–260. [PubMed: 16464738]
- Song X, Voronov E, Dvorkin T, Fima E, Cagnano E, Benharroch D, Shendler Y, Bjorkdahl O, Segal S, Dinarello CA, Apte RN. Differential effects of IL-1 alpha and IL-1 beta on tumorigenicity patterns and invasiveness. *J Immunol.* 2003; 15:6448–6456. [PubMed: 14662844]
- Sternberg EM, Hill JM, Chrousos GP, Kamilaris T, Listwak SJ, Gold PW, Wilder RL. Inflammatory mediator-induced hypothalamic-pituitary-adrenal axis activation is defective in streptococcal cell wall arthritis-susceptible Lewis rats. *Proc Natl Acad Sci USA.* 1989; 86:2374–2378. [PubMed: 2538840]
- Stevens RG. Light-at-night, circadian disruption and breast cancer: assessment of existing evidence. *Int J Epidemiol.* 2009; 38:963–970. [PubMed: 19380369]
- Strange KS, Kerr LR, Andrews HN, Emerman JT, Weinberg J. Psychosocial stressors and mammary tumor growth: an animal model. *Neurotoxicol Teratol.* 2000; 22:89–102. [PubMed: 10642118]
- Teunis MA, Kavelaars A, Voest E, Bakker JM, Ellenbroek BA, Cools AR, Heijnen CJ. Reduced tumor growth, experimental metastasis formation, and angiogenesis in rats with a hyperreactive dopaminergic system. *FASEB J.* 2002; 16:1465–1467. [PubMed: 12205050]
- Thompson HJ, Adlakha H. Dose-responsive induction of mammary gland carcinomas by the intraperitoneal injection of 1-methyl-1-nitrosourea. *Cancer Res.* 1991; 51:3411–3415. [PubMed: 2054781]
- Thompson HJ, Adlakha H, Singh M. Effect of carcinogen dose and age at administration on induction of mammary carcinogenesis by 1-methyl-1-nitrosourea. *Carcinogenesis.* 1992; 13:1535–1539. [PubMed: 1394836]
- Thompson HJ, McGinley JN, Rothhammer K, Singh M. Rapid induction of mammary intraductal proliferations, ductal carcinoma in situ and carcinomas by the injection of sexually immature female rats with 1-methyl-1-nitrosourea. *Carcinogenesis.* 1995; 16:2407–2411. [PubMed: 7586143]
- van den Biggelaar AH, Gussekloo J, de Craen AJ, Frolich M, Stek ML, van der Mast RC, Westendorp RG. Inflammation and interleukin-1 signaling network contribute to depressive symptoms but not cognitive decline in old age. *Exp Gerontol.* 2007; 42:693–701. [PubMed: 17350781]
- Voronov E, Shouval DS, Krelm Y, Cagnano E, Benharroch D, Iwakura Y, Dinarello CA, Apte RN. IL-1 is required for tumor invasiveness and angiogenesis. *Proc Natl Acad Sci USA.* 2003; 100:2645–2650. [PubMed: 12598651]

- Whalley B, Jacobs PA, Hyland ME. Correlation of psychological and physical symptoms with chronically elevated cytokine levels associated with a common immune dysregulation. *Ann Allergy Asthma Immunol.* 2007; 99:348–351. [PubMed: 17941282]
- Williams JB, Pang D, Delgado B, Kocherginsky M, Tretiakova M, Krausz T, Pan D, He J, McClintock MK, Conzen SD. A model of gene-environment interaction reveals altered mammary gland gene expression and increased tumor growth following social isolation. *Cancer Prev Res (Phila).* 2009; 2:850–861. [PubMed: 19789294]
- Yadav D, Sarvetnick N. Cytokines and autoimmunity: redundancy defines their complex nature. *Curr Opin Immunol.* 2003; 15:697–703. [PubMed: 14630205]
- Yee JR, Cavigelli SA, Delgado B, McClintock MK. Reciprocal affiliation among adolescent rats during a mild group stressor predicts mammary tumors and lifespan. *Psychosom Med.* 2008; 70:1050–1059. [PubMed: 18842748]
- Yoo SY, Lee SY, Yoo NC. Cytokine expression and cancer detection. *Med Sci Monit.* 2009; 15:RA49–RA56. [PubMed: 19247257]
- Zarbl H, Sukumar S, Arthur AV, Martin-Zanca D, Barbacid M. Direct mutagenesis of Haras-1 oncogenes by N-nitroso-N-methylurea during initiation of mammary carcinogenesis in rats. *Nature.* 1985; 315:382–385. [PubMed: 3923365]

Figure 1.

Pre-carcinogen plasma cytokine concentrations predict subsequent mammary tumor onset following carcinogen exposure. Correlation between weeks to tumor onset and (A) IL-1α, (B) IL-1β, and (C) TNFα. Median split analyses between early and late tumor onset were used to generate respective bar graphs. Single, linear regression coefficients (β) and R^2 values are listed for each panel. $n = 16$; $*p < 0.05$.

Figure 2.

Pre-carcinogen plasma corticosterone concentrations predict mammary tumor onset. Correlation between weeks to tumor onset and pre-carcinogen corticosterone concentrations. Median split analyses between early and late tumor onset were used to generate respective bar graphs. Single, linear regression coefficients (β) and R^2 values are listed on panel. $n = 19$; $^{**}p < 0.01$.

Figure 3.

Plasma pre-carcinogen cytokine concentrations negatively correlate with pre-carcinogen plasma corticosterone concentrations. Correlation between circulating pre-carcinogen corticosterone and pre-carcinogen (A) IL-1 β and (B) TNF α concentrations. Single, linear regression coefficients (β) and R^2 values are listed on panel. $n = 16$; $*p < 0.05$.

Figure 4.

Pre-carcinogen depressive-like behavior predicts subsequent mammary tumor onset. Correlation between weeks to tumor onset and percentage of floating behaviors using a real-time sampling method for the Porsolt forced swim test. A median split analysis between early and late tumor onset was used to generate the bar graph. The single, linear regression coefficient (β) and R^2 values is listed. Early onset: $n = 9$, late onset: $n = 9$; $*p < 0.05$.

Table 1

Linear regression used to predict pre-carcinogen corticosterone (independent variable) based on pre-carcinogen cytokine (dependent variable).

	β	R^2	p -Value
IL-1 α	-0.16	0.03	0.56
IL-1 β	-0.53	0.28	0.04*
TNF α	-0.60	0.36	0.01*

*
 $p < 0.05$.

Table 2

Multiple, linear regressions used to predict tumor onset (dependent variable) based on pre-carcinogen cytokine plus pre-carcinogen corticosterone (independent variables).

		<i>R</i> ²	<i>p</i> -Value	<i>b</i>	β	<i>p</i> -Value
Model 1	IL-1 α	0.31	0.09	-0.040	-0.49	0.06
	Corticosterone			0.009	0.20	0.40
Model 2	IL-1 β	0.39	0.04*	-0.024	-0.62	0.02*
	Corticosterone			-0.003	-0.06	0.81
Model 3	TNF α	0.30	0.10	-0.007	-0.58	0.07
	Corticosterone			-0.003	-0.07	0.81

* *p* < 0.05.