Psychoneuroendocrinology (2013) 38, 800—807

Available online at www.sciencedirect.com

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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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Received 12 June 2012; received in revised form 31 July 2012; accepted 3 September 2012

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

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-1α, IL-1β, TNFα) 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-1β 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.

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 depression (Dantzer et al., 2008; Leonard

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http://dx.doi.org/10.1016/j.psyneuen.2012.09.003

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 $\times$ 47.6 cm $\times$ 20.9 cm high) with a constant temperature and humidity of $22 \pm 1\ ^{\circ}C$ 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-ortibal 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