National Cancer Institute

# Biobehavioral Influences on Cancer Biology

## AN EMERGING OPPORTUNITY

U.S. DEPARTMENT
OF HEALTH AND
HUMAN SERVICES

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# National Cancer Institute

## Biobehavioral Influences on Cancer Biology: An Emerging Opportunity

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AN EMERGING OPPORTUNITY

# Biobehavioral Influences on Cancer Biology: An Emerging Opportunity

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Clinical and epidemiological observations of biobehavioral[a] processes and cancer risk have historically yielded inconsistent results. As our understanding of cancer biology has deepened, the basis for mixed findings has become more apparent. Cancer is now understood not to be a single homogenous disease entity, but a collection of individual pathologies with distinctive properties that vary according to the tissue of origin and the specific constellation of genomic alterations driving malignant cell growth in each individual tumor.

We also now recognize that much of cancer's disease burden stems from mechanisms of tumor progression (e.g., angiogenesis, invasion, metastasis), which may be distinct from the biological processes initiating neoplastic growth (e.g., genomic damage, inflammation, growth factors, and other physiologic proliferation signals). Stronger epidemiological associations have been observed between biobehavioral processes and cancer progression, rather than cancer incidence. The biologic mechanisms that may account for such observations are being discovered through the convergence of relevant molecular, cellular, and clinical data.

A novel transdisciplinary framework for understanding the influence of biobehavioral processes (e.g., depression, social isolation, and chronic distress) on the clinical course of neoplastic disease is emerging. Advances in the fields of neuroendocrinology, cancer biology, behavioral science, stress physiology, and bioinformatics are leveraged to identify relevant physiologic signaling pathways and new therapeutic targets.

Biobehavioral factors such as depression, social isolation, and chronic stress have long been anecdotally linked to cancer onset, but mechanistic research in this area diminished following mixed results from human epidemiologic studies of cancer incidence. However, recent findings of direct neuroendocrine control of tumor biology are providing new insights into the basis for biobehavioral influences in cancer, and suggest novel opportunities for adjuvant control of tumor progression. This chapter summarizes the historical development of science on biobehavioral influences on cancer and provides a

*Recent findings of direct neuroendocrine control of tumor biology are providing new insights into the basis for biobehavioral influences in cancer and suggest novel opportunities for adjuvant control of tumor progression.*

[a] Study of the interaction between social, psychological, and biological factors in health.

framework for considering the emerging opportunities in this area.

### Biobehavioral Signaling Pathways

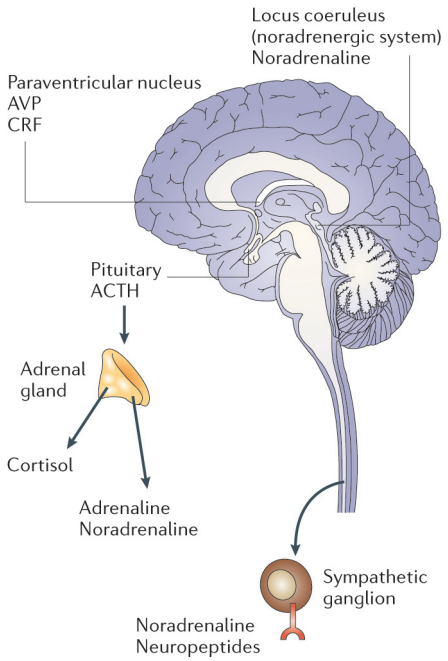
Biobehavioral influences can be distinguished from strictly behavioral risk factors such as smoking by the key involvement of the brain in interpreting social experience and subsequently regulating systemic physiology via neuroendocrine activity.

Environmental and psychological processes initiate a cascade of information-processing pathways in the central nervous system and periphery, which activate the autonomic nervous system (ANS) or the hypothalamic-pituitary-adrenal (HPA) axis (Figure 1).[1,2] Cognitive and emotional feedback from cortical and limbic areas of the brain modulate the activity of hypothalamic and brain stem structures directly controlling ANS and HPA activity. HPA responses are mediated by hypothalamic production of corticotrophin-releasing factor (CRF) and arginine vasopressin, which activate the secretion of pituitary hormones such as adrenocorticotrophic hormone (ACTH), enkephalins, and endorphins. ACTH induces downstream release of glucocorticoids such as cortisol from the adrenal cortex.

Glucocorticoids control growth, metabolism, and immune function, and have a pivotal role in regulating basal function and stress reactivity across a wide variety of organ systems. ANS responses to stress are mediated primarily by activation of the sympathetic nervous system (SNS), and subsequent release of catecholamines (principally norepinephrine and epinephrine) from sympathetic neurons and the adrenal medulla. Individual differences in the perception and evaluation of external events (appraisal and coping processes) create variability in ANS and HPA activity levels. Stressors that have been associated with alterations in neuroendocrine dynamics include marital disruption, bereavement, depression, chronic sleep disruption, severe trauma, and post-traumatic stress disorder.

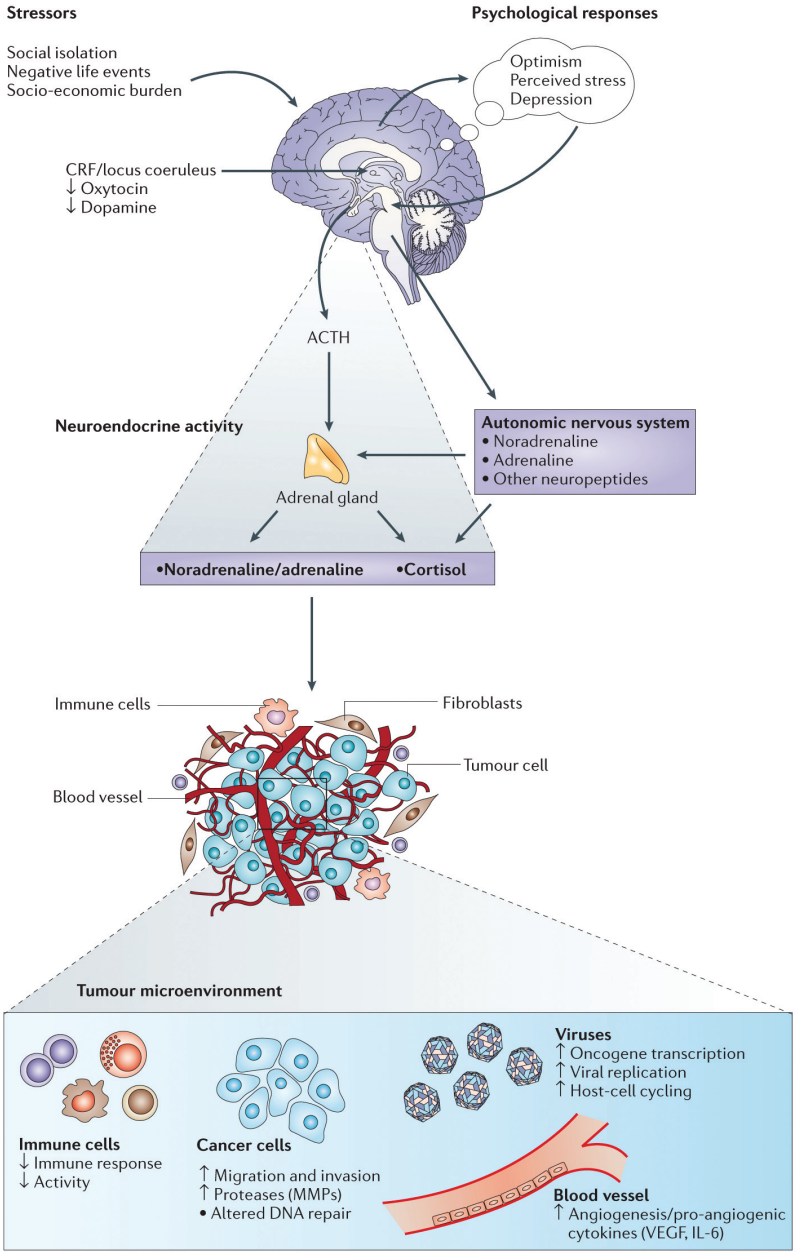
Recent developments in our molecular understanding of cancer biology and the tumor microenvironment provide a new framework for understanding the impact of biobehavioral risk factors such as stress and social isolation on the clinical course of neoplastic disease (Figure 2, p. 3).

**FIGURE 1** Important components of the central and peripheral stress systems




Stressful experiences activate components of the limbic system, which includes the hypothalamus, the hippocampus, the amygdala, and other nearby areas. In response to neurosensory signals, the hypothalamus secretes corticotrophin-releasing factor (CRF) and arginine vasopressin (AVP), both of which activate the pituitary to produce hormones such as adrenocorticotropic hormone (ACTH). Circulating ACTH stimulates the production of glucocorticoids from the adrenal cortex. The sympathetic nervous system originates from the brainstem, and the preganglionic neurons terminate in the ganglia near the spinal column. From these ganglia, postganglionic fibers run to the effector organs. The main neurotransmitter of the pre-ganglionic sympathetic fibers is acetylcholine and the typical neurotransmitter released by the post-ganglionic neurons is noradrenaline. The adrenal medulla contains chromaffin cells, which release mainly adrenaline. Reprinted with permission.

**FIGURE 2** Effects of stress-associated factors on the tumour microenvironment




The responses to stressors involve central nervous system (CNS) perceptions of threat and subsequent activation of the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal (HPA) axis. Catecholamines, glucocorticoids and other stress hormones are subsequently released from the adrenal gland, brain and sympathetic nerve terminals and can modulate the activity of multiple components of the tumour microenvironment.

Effects include the promotion of tumour-cell growth, migration and invasive capacity, and stimulation of angiogenesis by inducing production of pro-angiogenic cytokines. Stress hormones can also activate oncogenic viruses and alter several aspects of immune function, including antibody production, cytokine production profiles and cell trafficking.

Collectively, these downstream effects create a permissive environment for tumour initiation, growth and progression. CRF, corticotrophin-releasing factor; *IL-6*, interleukin-6; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor. Reprinted with permission.

Activation of such biobehavioral signaling pathways can lead to alterations in nuclear, cellular, or organ system function and/or structure. We hypothesize that these changes can be characterized at molecular, cellular, or physiologic levels and measured grossly within the tumor microenvironment. This systemic regulation includes opportunities to modulate the biology of tumor cells and their tissue microenvironment. As such, biobehavioral dynamics can be construed as a tumor "macroenvironment" that may structure multiple aspects of cancer biology, and thus provide a leveraged opportunity to affect the course of disease.

## The Epidemiology of Biobehavioral Influences on Cancer

Hypothesized associations between cancer and stressful life events, personality characteristics, and emotions have existed since the second century AD when Galen proposed that melancholic women were more susceptible to cancer than women who were sanguine.[3,4]

More recently, mixed epidemiological data suggest that psychological and social characteristics might be associated with differential cancer onset, progression, and mortality.[2, 5-12] However, findings have been inconsistent and historically this body of research has been challenged by methodological constraints.[13,14] Heterogeneous results can be attributed to differences in type of cancer, stressor, and outcome studied, length of follow-up, and control for confounding and/or clinical correlates of disease.[6,14,15] Retrospective and case control studies have suggested positive associations between stressful life events and cancer risk, whereas, prospective studies tend to show no association.[6,16]

In general, stronger relationships have been observed between psychosocial factors and cancer progression than between psychosocial factors and cancer incidence (for a discussion of the strengths and weaknesses of this literature, see references 14 and 17). Data from patients with existing tumors show that cancer diagnosis and treatment cause substantial distress. Those who tend toward depressive coping methods, such as hopelessness and helplessness, might experience accelerated disease progression.[18] By contrast, positive factors such as social support and optimism have predicted longer survival,[19,20] but the influence of psychosocial factors on survival remains uncertain.[14]

## Leveraging Resources and Knowledge Facilitates an Emerging Opportunity

As our understanding of cancer biology matures,[21,22] the basis for the mixed findings on biobehavioral risk factors for cancer has become more apparent. Cancer is now understood not to be a single homogenous disease entity, but a collection of individual pathologies with distinctive properties that vary according to the tissue of origin and the specific constellation of genomic alterations driving malignant cell growth in each individual tumor.[23-26]

It is plausible that biobehavioral factors might influence cancer differentially depending on the specific biology of a particular tumor. We also now recognize that much of cancer's disease burden stems from mechanisms of tumor progression (e.g., angiogenesis, invasion, metastasis), which may be distinct from the biological processes initiating neoplastic growth (e.g., genomic damage, inflammation, growth factors, and other physiologic proliferation signals).[21] This more complete and nuanced view of cancer biology provides a framework for understanding why biobehavioral dynamics might exert varying effects across different tumor types, or at different stages of disease.

A series of studies in the 1960s and 1970s showed that experimentally imposed stress could influence cancer onset and progression in animal models of virally induced cancer.[27-31]

This research waned as several negative epidemiologic findings emerged in the human literature and the focus of cancer biology moved away from viral etiology and immune surveillance as major determinants of human cancer risk. Today, the increasing mechanistic sophistication and relevancy of animal models of human cancers[32,33] offer methodological opportunities to advance the science of biobehavioral influences of cancer biology.

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The emergence of immunodeficient mouse models involving implanted human tumor cells provides a framework for evaluating behavioral influences on aspects of cancer biology that more accurately recapitulates the dynamics of human cancer *in vivo*. This opportunity has recently been exploited to demonstrate direct effects of stress and social isolation on the biology of human tumor cells (i.e., independent of any effects mediated by immune modulation[34]). These more accurate animal models of human cancer biology provide a context for experimental manipulation of behavioral factors (isolation, stress, depression) and discovery of the consequent changes in brain function, neuroendocrine activity, and peripheral biology that impact tumor cells and their microenvironment.

The growing availability of transgenic and knockout mouse models of cancer provides additional opportunities to understand how biobehavioral factors and the neuroendocrine system can impact the molecular biology of cancer.

We have improved our conceptual understanding of stress and have moved from broad nonspecific measures to the consideration of refined psychological constructs like subjective distress, depression, social isolation, social support, and helplessness/hopelessness.[14,35] We understand the connection between perception and interpretation of stressors and the concomitant physiological response. Furthermore, we recognize the importance of appropriate measurement of relevant stress mediators at and within the tumor microenvironment, rather than from the periphery.[6,36–38]

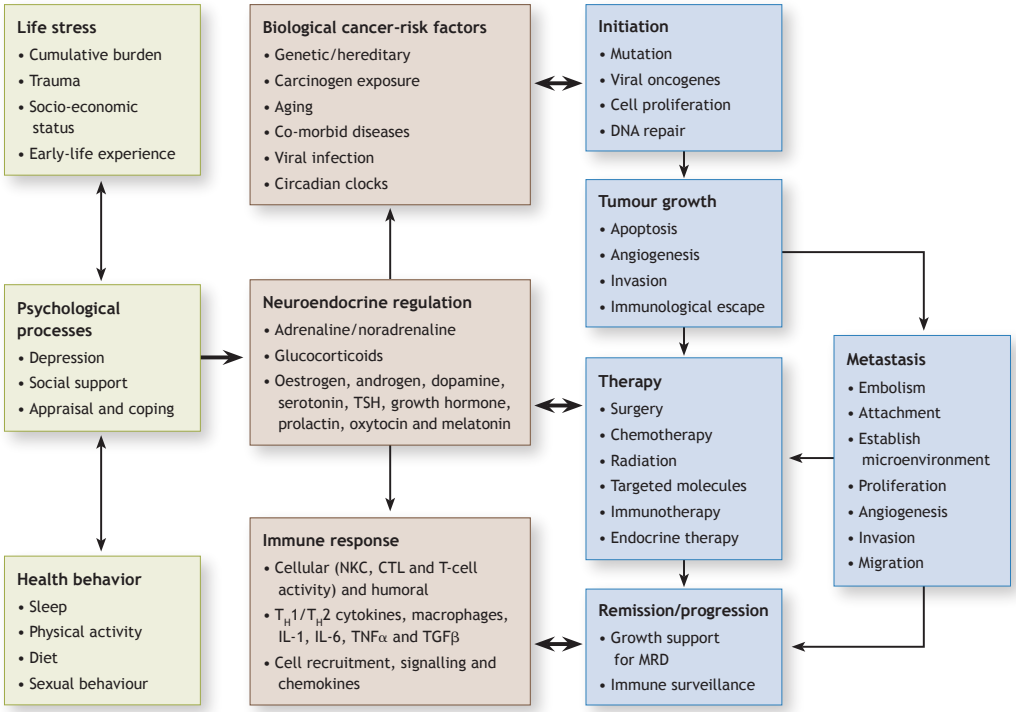
A small number of human studies have identified biological correlates of psychosocial risk that potentially influence tumor cell biology. For example, within the ovarian cancer context, depression and social support have been linked to circulating VEGF levels *in vivo* and to catecholamine regulation of tumor cell VEGF production *in vitro*.[37,39,40]

Emerging data from experimental animal models[34,41–44] and clinical and epidemiological studies[7,9,17,18,37,40,45] have revitalized empirical interest in whether perceptions, cognitions, affective states, social interactions, and objective experiences that trigger mediators of the stress response (e.g., catecholamines, glucocorticoids, and inflammatory markers) affect tumor processes, either directly or as a part of a chain of events. The biologic mechanisms that may account for such observations are being discovered through the convergence of relevant molecular, cellular, and clinical data.

### Implications

As our understanding of the biologic and clinical significance of neuroendocrine-mediated influences on cancer pathogenesis

**FIGURE 3** Integrated model of bio-behavioural influences on cancer pathogenesis through neuroendocrine pathways




In this model, biobehavioural factors such as life stress, psychological processes and health behaviours (green panel) influence tumour-related processes (blue panel) through the neuroendocrine regulation of hormones, including adrenaline, noradrenaline and glucocorticoids (brown panel). Central control of peripheral endocrine function also allows social, environmental and behavioural processes to interact with biological risk factors such as genetic background, carcinogens and viral infections to systemically modulate malignant potential (brown panel).

Direct pathways of influence include effects of catecholamines and glucocorticoids on tumour-cell expression of genes that control cell proliferation, invasion, angiogenesis, metastasis and immune evasion (blue panel). Stress-responsive neuroendocrine mediators can also influence malignant potential indirectly through their effects on oncogenic viruses and the cellular immune system (brown panel). These pleiotropic hormonal influences induce a mutually reinforcing system of cellular signals that collectively support the initiation and progression of malignant cell growth (blue panel).

Furthermore, neuroendocrine deregulation can influence the response to conventional therapies such as surgery, chemotherapy and immunotherapy (blue panel). In addition to explaining bio-behavioural risk factors for cancer, this model suggests novel targets for pharmacological or behavioural intervention. CTL, cytotoxic T lymphocytes; *IL*, interleukin; MRD, minimal residual disease; NKC, natural killer cell; TGFβ, transforming growth factor-β; TNFα, tumour-necrosis factor-α; TSH, thyroid-stimulating hormone. Reprinted with permission.

expands consideration of novel therapeutic paradigms that integrate a biobehavioral perspective is warranted. Successful management of factors such as stress and negative mood might have a salubrious effect on the neuroendocrine regulation of oncogenesis, tumor growth and metastasis, and cancer immunoediting processes. Despite significant progress within the past decade, further research is needed to define the mechanisms underlying the complex circuits involving the HPA and ANS axes and their effects on the processes involved in cancer development and progression.

It is important to note that stress per se does not cause cancer; however, clinical and experimental data indicate that stress

and other factors such as mood, coping mechanisms, and social support can influence the underlying cellular and molecular processes that facilitate the progression of malignant cell growth.[1,37,39,40]

*It is important to note that stress per se does not cause cancer; however, clinical and experimental data indicate that stress and other factors such as mood, coping mechanisms, and social support can influence the underlying cellular and molecular processes that facilitate the progression of malignant cell growth.*

The evolving discovery of neuroendocrine-mediated influences on cancer biology provides a unique opportunity to consider additional influences on the clinical course of cancer. Consideration of this perspective may potentially contribute to the development of novel therapeutic interventions to preempt cancer at every opportunity.

The research featured in this report supports a model in which biobehavioral factors influence multiple aspects of tumorigenesis via their impact on neuroendocrine function (Figure 3, p. 6). This research program exemplifies four key forces—convergence, integrative approaches, connectivity, and the leveraging of resources and knowledge—"that drive the promise and hope for a better world where cancer is preempted and the best outcomes are assured for all."[46] This science contributes to the National Cancer Institute's (NCI) objective to "understand the causes and mechanisms of cancer" and highlights some of NCI's most promising investments in biobehavioral research related to cancer control. Furthermore, this research has the potential to contribute to the development of effective and efficient adjuvant and complementary treatments to ensure that best outcomes are achieved for all.

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# Effects of Chronic Stress on Cancer Growth and Progression

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Over 25 years ago, George L. Engel recognized that biological factors alone cannot account for all changes in physical health and that social and behavioral dimensions must also be considered.[1] In clinical and epidemiological studies, cancer progression and, to a lesser extent, cancer onset have been related to chronic stress, depression, lack of social support, and other psychological factors.[2–6]

Stress is a complex process encompassing environmental and psychosocial factors and initiates a cascade of information-processing pathways in both the central and peripheral nervous systems. Ultimately the fight-or-flight stress responses in the autonomic nervous system (ANS) or the defeat/withdrawal responses in the hypothalamic-pituitary-adrenal axis (HPA) are generated and secrete catecholamines and cortisol, respectively (see figure on page 2).[7,8] Activation of these pathways in acute stress is necessary for survival and reflects adaptive processes. In contrast, chronic stress negatively affects most physiological systems due to prolonged exposure to catecholamines and glucocorticoids.[9]

*Emerging research is beginning to explore the role of neuropeptides and neurotransmitters, which are increased in certain biobehavioral states, on the multistep process of cancer metastasis.*

Chronic stress has been shown to decrease cellular immune parameters, such as natural killer (NK) cell cytotoxicity and T-cell responses to mitogen stimulation.[10–12] Effects of biobehavioral factors on the immune system are thought to be mediated in part by the sympathetic nervous system, the HPA axis, and a variety of other hormones and peptides.[13, 14]

To date, the majority of neuroendocrinological research dealing with stress and accelerated tumor growth has focused on suppressed immune response to malignant tissue.[7] Recently, we and others have considered other biological pathways that may be affected by stress mediators. These observations are the focus of the current review.

## Neuroendocrine Influences on Cancer

Tumorigenesis is a multistep process. According to Hanahan and Weinberg,[15] there are six essential, acquired alterations in cell physiology that promote malignant growth:

- Self-sufficiency in growth signals,
- Insensitivity to anti-growth signals,
- Evasion of apoptosis,
- Limitless replicative potential,
- Sustained angiogenesis, and
- Tissue invasion and metastasis.

After a cell acquires tumorigenic potential, cancer metastasis can occur if another series of sequential interrelated steps occur, including:

1. Proliferation/angiogenesis,
2. Invasion,
3. Embolism/circulation,
4. Transport,
5. Adherence in organs,
6. Adherence to vessel wall, and
7. Extravasation.[16]

Tumor progression is a result of crosstalk between different cell types within the tumor and its surrounding microenvironment.[17] Emerging research is beginning to explore the role of neuropeptides and neurotransmitters, which are increased in certain biobehavioral states, on the multistep process of cancer metastasis.

*There is growing evidence that stress hormones may affect tumor cell motility and invasion.*

In order to proliferate, tumor cells rely on nutrient and oxygen diffusion. The effects of stress-related hormones on tumor cell proliferation can be either stimulatory or inhibitory, depending on the type of hormone and tumor type. For example, in breast carcinoma, activation of β-adrenergic receptors (ADRB) has been associated with accelerated tumor growth.[18–20] In contrast, catecholamines may inhibit tumor cell proliferation that may be mediated by α-adrenergic receptors or the dopamine transporter. Scarparo and colleagues found that melanoma cells treated with the α1–adrenergic agonist phenylephrine led to a dose-dependent decrease in proliferation, which could be reversed by the α1–adrenergic antagonist prazosin.[21] Additionally, norepinephrine treatment shifted neuroblastoma cells expressing the dopamine transporter into the $G_0/G_1$ phase, thereby inhibiting proliferation.[22] Similarly, the role of glucocorticoid hormones on proliferation is dual.[23,24]

The ability of a tumor cell to invade and metastasize to distant tissues is highly dependent on malignant cell adhesion to the extracellular matrix.[25] Enserink and colleagues have shown that the β-agonist isoproterenol promotes ovarian cancer cell spread and adhesion via integrins through the Epac (exchange factor directly activated by cAMP)-Rap1 pathway.[26,27] Additionally, there is growing evidence that stress hormones may affect tumor cell motility and invasion. Norepinephrine has been shown to induce breast and colon cancer migration.[28,29] We have previously demonstrated that physiologic stress concentrations of norepinephrine and epinephrine can enhance the invasive potential of ovarian carcinoma cells via the ADRB-mediated increases in matrix metalloproteinases (MMPs). The β-adrenergic antagonist propranolol and pharmacologic blockade of MMPs abrogated the effects of norepinephrine on the increases in tumor cell invasive potential.[30] This work provided *in vitro* evidence that stress hormones can increase the invasive potential of ovarian cancer cells.

Avoidance of apoptosis is a critical component of the metastatic cascade. Thus far, glucocorticoids, which regulate a variety of cellular processes, have been the focus of research elucidating the role of stress hormones on tumor cell survival. Glucocorticoids downregulate proapoptotic elements of the death receptor and mitochondrial apoptosis pathways in cervical and lung cancer cell lines.[31] Wu and colleagues found that breast cancer

cell lines pretreated with dexamethasone inhibited chemotherapy-induced apoptosis via transcriptional induction of serum and GC-inducible protein kinase-1 (SGK-1) and mitogen activated protein kinase phosphatase-1 (MKP-1).[32] The antiapoptotic effects of glucocorticoid treatment could be reversed by a blockade of SGK-1 and MKP-1.[32] Additionally, glucocorticoids and catecholamines may act synergistically to facilitate cancer growth, as evidenced in lung carcinoma cell lines.[33]

*Propranolol, a nonspecific β-blocker, completely blocked the effects of immobilization stress on tumor growth, indicating a critical role for β-adrenergic signaling in stress mediated increases in tumor growth.*

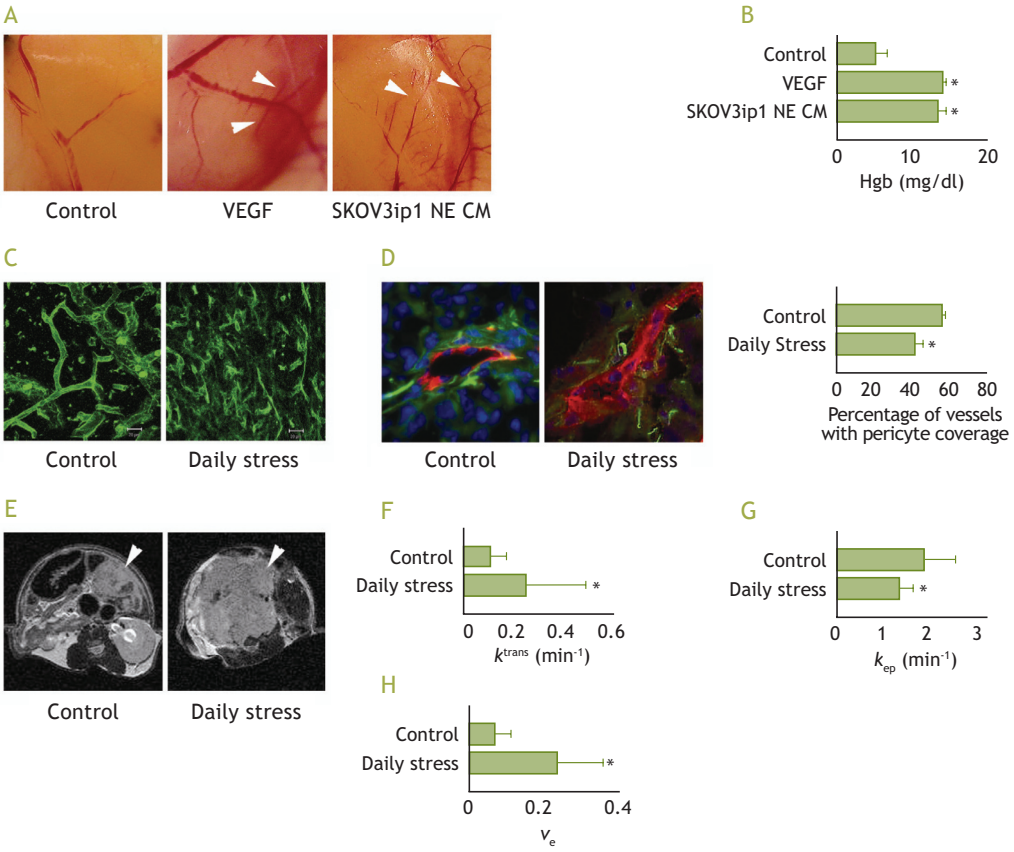
Angiogenesis is a key process in the growth of most solid tumors beyond 1–2 mm in diameter, and their metastatic spread involves recruitment of nearby blood vessels to permeate the tumor.[34] In vascular endothelial cells, ischemic neoangiogenesis causes proliferation via overexpression of the ADRB.[35] Vascular endothelial growth factor (VEGF) is a key proangiogenic cytokine that is produced by tumor cells, endothelial cells, and platelets.[36] We have previously reported that higher levels of social support were correlated with lower VEGF levels in serum from presurgical patients with ovarian carcinoma providing a possible mechanism by which poor social support may be associated with disease progression.[37] We have also demonstrated that VEGF production by ovarian cancer cell lines was enhanced by stress hormones such as norepinephrine, epinephrine, and isoproterenol *in vitro* and blocked by the β-antagonist, propranolol.[38] Based on our previous studies, we sought to elucidate whether chronic stress and the associated increase in sympathetic nervous system activity had a causal effect on growth and metastasis of ovarian cancer *in vivo*.[39]

### The Role of Chronic Stress on Tumor Growth and Angiogenesis in Orthotopic Ovarian Carcinoma

We recently demonstrated that chronic stress (daily restraint) quantitated by elevated organ catecholamine (norepinephrine) and cortisol levels enhanced the pathogenesis of ovarian carcinoma *in vivo*, as evidenced by increased tumor weight and more invasive pattern of metastasis including parenchymal liver, spleen, and diaphragm involvement. Propranolol, a nonspecific β-blocker, completely blocked the effects of immobilization stress on tumor growth, indicating a critical role for β-adrenergic signaling in stress mediated increases in tumor growth. The β-adrenergic receptors are G-protein-coupled receptors that mainly function to transmit extracellular information to the interior of the cell, causing an activation of adenylyl cyclase and an accumulation of the second messenger cAMP to activate the protein kinase A pathway.[40] Ultimately, after catecholamine stimulation, the activation of the tumor cell cAMP-protein kinase A signaling pathway led to increased VEGF gene expression, resulting in increased tumor vascularization and more aggressive growth.

A series of experiments using either ADRB-null cell lines, pharmacological β-agonists, or ADRB-silencing with siRNA, demonstrated that ADRB2 on the tumor cells plays a functionally significant role in stress-mediated angiogenesis. The increased angiogenesis occurred in response to increases in catecholamine induced VEGF production by tumor cells. The tumor

**FIGURE 1** Effects of chronic stress on angiogenesis




**(A)** Representative images of Matrigel containing either serum-free SKOV3ip1 conditioned medium (control), VEGF (0.25 nM), or serum-free conditioned medium from norepinephrinetreated SKOV3ip1 cells (SKOV3ip1 NE CM). Newly formed blood vessels in the Matrigel are indicated by arrowheads. **(B)** We excised the Matrigel plugs and used them for quantification of angiogenesis by measuring the hemoglobin (Hgb) content in the Matrigel matrix. *P < 0.01 versus control. **(C)** We visualized tumor vasculature from HeyA8 tumors in control and stressed animals by confocal fluorescence microscopy after perfusion with FITC-lectin. **(D)** We examined vessel maturation by determining the extent of pericyte coverage in tumors from control and chronic stress-exposed animals using triple immunofluorescence (CD31 (red) for endothelial cells, desmin (green) for pericytes and Hoechst (blue) for nuclei). *P < 0.01. **(E)** Magnetic resonance imaging (MRI) was used to evaluate the functional characteristics of the tumor vasculature. Representative *in vivo* axial images of intraperitoneal HeyA8 ovarian tumors (arrowheads) from control and stressed animals were acquired in a 4.7-T scanner using a T2-weighted fast spin echo sequence (FSE) and respiratory gating (day 18 post-inoculation with HeyA8 cells). Vascular parameters derived from dynamic contrast–enhanced MRI included **(F)** $K^{trans}$, the volume transfer constant between blood plasma and extravascular extracellular space, **(G)** $K_{ep}$, the rate constant between extravascular extracellular space and blood plasma and **(H)** $v_e$, the volume of extravascular extracellular (interstitial) space per unit volume of tissue. We made measurements from the enhancing periphery of each tumor (n=8 per group; *P < 0.05). Error bars represent s.e.m. Reprinted with permission.

vasculature in stressed animals contained more tortuous and numerous blood vessels than controls (Figure 1), and was accompanied by a significant decrease in the proportion of blood vessels with pericyte coverage in tumors from stressed animals, which suggests more immature vasculature. Additionally, magnetic resonance imaging and kinetic analysis of the stressed tumors showed substantial anatomical and functional alterations in tumor vasculature.

Both propranolol and VEGF-blocker such as the VEGF-R2 inhibitor PTK787 or the monoclonal VEGF-specific antibody bevacizumab completely blocked the stress induced effects on tumor burden and invasiveness (Figure 2, p. 15).

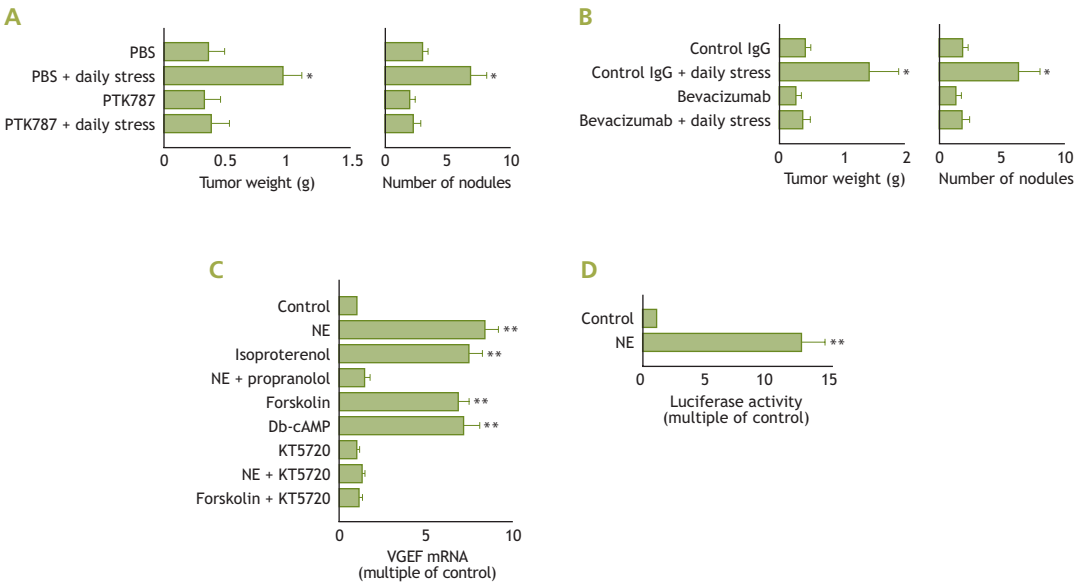
*The tumor vasculature in stressed animals contained more tortuous and numerous blood vessels than controls.*

These results demonstrated that behavioral stressors can enhance the pathogenesis of ovarian carcinoma via VEGF-mediated angiogenesis *in vivo* (Figure 3, p. 16), and underscores the importance of the neuroendocrine system in cancer pathogenesis.

## Clinical Opportunities and Challenges

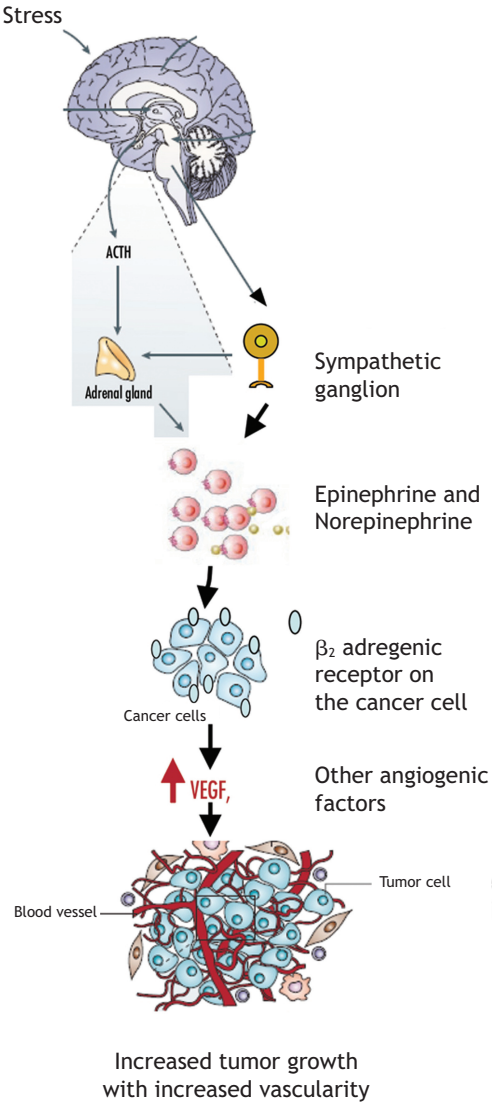
The knowledge of how stress biology affects tumor initiation and pathogenesis is gradually expanding. Biobehavioral factors such as chronic stress, depression, and social support have been linked to tumor biology via their endocrine consequences and cell mediated immunity. Experimental studies are ongoing that will dissect the molecular signaling pathways responsible for mediating the effects of stress on cancer growth and progression. Although these pathways have not been fully elucidated, **the studies to date indicate possible opportunities for behavioral and pharmacological intervention that target tumor-supporting neuroendocrine dynamics.**

**FIGURE 2** Effect of VEGF on stress-induced tumor growth




**(A)** HeyA8-injected mice were randomly assigned to one of the following four groups (4 d after tumor cell injection; n=10 per group): control, oral placebo with stress, 50 mg/kg oral PTK787 (VEGF-R2 inhibitor) daily with no stress, or PTK787 with stress. **(B)** HeyA8-injected mice were randomly assigned to one of the following four groups (4 d after tumor cell injection; n=10 per group): control, oral placebo with stress, bevacizumab (VEGF-specific antibody; 5 mg/kg intraperitoneally, twice per week) with no stress, or bevacizumab with stress. Treatment with PTK787 or bevacizumab blocked the stress-induced increase in tumor weight and number of nodules compared with treatment with the oral placebo (PBS) plus stress. **(C)** VEGF mRNA increased significantly when SKOV3ip1 cells were stimulated with norepinephrine (1 μM), isoproterenol (1 μM), forskolin (activator of cAMP) (1 μM), or dibutyryl cAMP (db-cAMP) (1 μM). KT5720 (1 μM) is a selective inhibitor of the cAMP-dependent protein kinase A and blocked VEGF induction by norepinephrine and forskolin. **(D)** In SKOV3ip1 cells, the VEGF promoter activity was increased by 1,280% after norepinephrine treatment compared with vehicle control. *P ≤ 0.01; **P ≤ 0.001. Reprinted with permission.

**FIGURE 3** Effects of stress on ovarian cancer growth via VEGF-mediated angiogenesis




Chronic stress leads to the activation of the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis, which results in release of catecholamines such as norepinephrine and epinephrine. These catecholamines then activate the β-adrenergic receptors on ovarian cancer cells to secrete VEGF and other angiogenic factors, leading to enhanced tumor growth and increased vascularity. Reprinted with permission from *Cell Cycle* (In Press).

Pharmacologic and genetic manipulations identify β-adrenergic signaling as a central mediator of stress effects on cancer growth; therefore, pharmacological interventions such as β-blockers potentially could be used to alleviate the effects of stress on cancer growth and progression. Interestingly, in a large case-control study of prostate cancer patients, Perron and colleagues found that among individuals taking antihypertensive medications, only β-blockers were associated with a reduction in cancer risk.[41]

A cohort study of cardiovascular patients that used β-blockers had a 49% decrease in cancer risk compared to patients that never used β-blockers. Moreover, there was a 6% decrease in risk for every additional year of β-blocker use.[42] However, other population-based, case-control studies of breast carcinoma patients have not confirmed alterations in risk with the use of β-blockers.[43,44] The efficacy of β-blockers in blocking the stress-mediated effects on tumor progression remains to be examined.

To the extent that behavioral and central nervous system processes modulate the activity of multiple hormones[45–48] and those processes are linked to angiogenic parameters in human clinical studies,[37,49] interventions targeting neuroendocrine function at the CNS level might also represent novel strategies for protecting cancer patients from the detrimental effects of stress biology on the progression of malignant disease. Such interventions may include behavioral interventions alone or in combination with pharmacological approaches.[8]

## Conclusions

Although research has shown that stress hormones affect tumor pathogenesis at multiple levels (initiation, tumor growth, and metastasis), our understanding of the underlying mechanisms is in its infancy and needs to be expanded. Based on the importance of the interplay between immunological and behavioral factors

*Interventions targeting neuroendocrine function at the level of the central nervous system could represent a novel strategy for protecting cancer patients from the deleterious effects of stress biology on cancer progression.*

providing a favorable microenvironment for tumor initiation and growth, there is a crucial need to integrate a biobehavioral perspective in therapeutic paradigms of human carcinoma. Interventions targeting neuroendocrine function at the level of the central nervous system could represent a novel strategy for protecting cancer patients from the deleterious effects of stress biology on cancer progression. Theoretically, these pharmacologic and behavioral interventions can be used concomitantly with conventional therapies to maximize efficacy and warrant further study, especially as cancer treatment evolves to encompass more patient-specific therapeutic approaches.

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## Anil K. Sood, MD

Dr. Sood joined the Department of Gynecologic Oncology at the University of Texas, M.D. Anderson Cancer Center in 2002 and is a physician scientist and Director of Ovarian Cancer Research. He has a joint appointment in the Department of Cancer Biology and maintains a research laboratory where his research focuses on mechanisms of cancer invasion and metastasis in ovarian cancer. Specifically, his research is focused in three main areas: 1) effect of neuroendocrine stress hormones on ovarian cancer growth and progression; 2) development of novel anti-vascular therapeutic approaches; and 3) development of new strategies for in vivo siRNA delivery. Dr. Sood has published numerous peer-reviewed articles and has authored and co-authored several book chapters. His research is funded by the National Cancer Institute and the Department of Defense. Dr. Sood has received major recognition for his research accomplishments including the Hunter Award from the American Gynecological, and Obstetrical Society, the James Nolan research award and the Gynecologic Cancer Foundation/Margaret Greenfield/Carmel Cohen, MD, Excellence in Ovarian Cancer Research Award. He has been invited to give presentations at many national and international forums.

Dr. Sood currently serves on the editorial board for several journals and is a reviewer for many others. He has served on study sections for the National Institutes of Health and the Department of Veterans Affairs Merit Review Grants. He is a Fellow in the American College of Obstetricians and Gynecologists and a Fellow in the American College of Surgeons. He has served and holds membership in several national organizations including the American Association for Cancer Research, American Society of Clinical Oncology, International Gynecologic Cancer Society, Society of Gynecologic Oncologists, Psycho-Neuro-Immunology Research Society, and the Western Association of Gynecologic Oncologists. He is a member of the Gynecologic Oncology Group's Committee on Experimental Medicine.

Dr. Sood is actively involved in teaching graduate students and clinical fellows. He is a member of The University of Texas Graduate School of Biomedical Sciences and teaches classes related to cancer cell signaling pathways. He was the recipient of M. D. Anderson's Outstanding Educator Award in 2003 and the Educator of the Month Award in 2005.

# Social Environment and Tumor Biology: The Role of Glucocorticoid-Mediated Tumor Cell Survival

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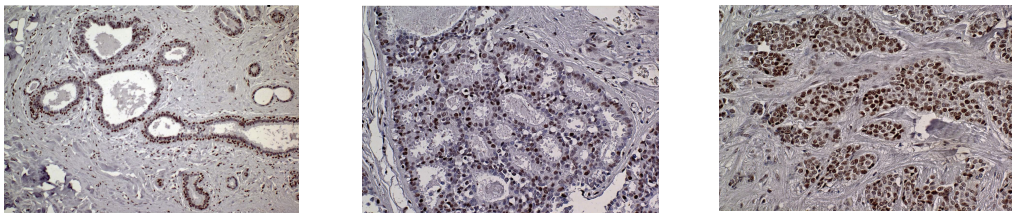
Individual differences in social conditions that occur early in life and remain stable through development exert potentially powerful effects on physical health. Research in our laboratory explores the connection between the psychosocial environment and mammary gland cancer development. The main goal of our studies is to determine whether psychosocial factors influence breast tumor biology, and if so, to characterize the physiological and molecular mechanisms through which these environmental factors act. My laboratory has a long-standing interest in glucocorticoid action in mammary epithelial cell biology.

Through interdisciplinary collaborations created in The University of Chicago's Center for Interdisciplinary Health Disparities Research, one of the NCI-funded Centers for Population Health and Health Disparities (CPHHD), we are now using a mouse model of breast cancer to study the effect of chronic social isolation on the corticosterone response to acute stress, as well as the biological mechanisms whereby altered corticosterone responses may affect mammary gland cancer development. In humans it is nearly impossible to accurately chart the lasting impact of early psychosocial influences on distant pathological outcomes such as breast cancer. However, animal models allow an exciting opportunity to observe how psychosocial events may result in physiological changes, and in turn, in changes in gene expression and ultimately, tumor development.

*Animal models allow an exciting opportunity to observe how psychosocial events may result in physiological changes and, in turn, in changes in gene expression and ultimately, tumor development.*

Glucocorticoids are well-known for their anti-inflammatory and immunosuppressive properties, as well as for their essential role in embryonic development. The majority of the properties of glucocorticoids are thought to be a consequence of the ability of the activated glucocorticoid receptor (GR) to act as a transcription factor, either through its DNA-binding-dependent mechanism or through cross-talk and often interference with other transcription factors. Although the GR is expressed ubiquitously in normal human mammary epithelium as well as in some human breast cancers, the role of the receptor in mammary epithelial cell (MEC) biology has received relatively little attention (Figure 1, p. 22). *In vitro*, a physiological stress dose concentration of hydrocortisone ($10^{-6}$ M) has long been added to the mixture of survival and growth factors required for successful epithelial cell culture in serum-free conditions.

**FIGURE 1** Human normal mammary epithelium, ductal carcinoma *in situ*, and invasive carcinomas express the glucocorticoid receptor



Normal mammary epithelium | Ductal carcinoma *in situ* | Invasive breast cancer

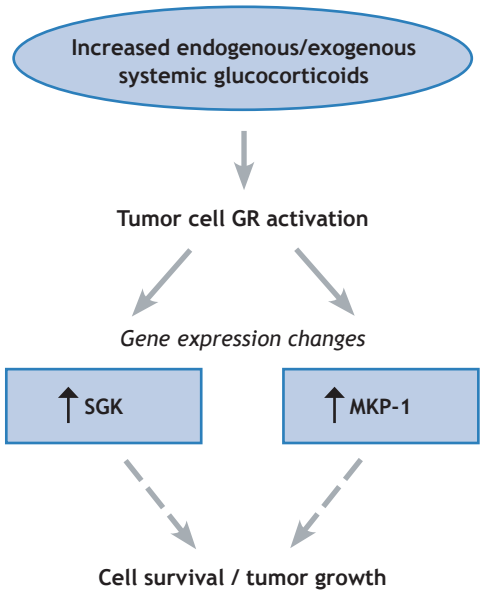
The glucocorticoid receptor is detected by immunohistochemistry at 200X magnification.

Furthermore, the importance of glucocorticoids for optimal plating efficiency of mammary epithelial cells has suggested a possible role in cell survival. The molecular basis of this observation has been addressed as described below, using large-scale gene arrays to examine changes in gene expression after glucocorticoid receptor (GR) activation. **Surprisingly, we found that GR activation was the single most important growth factor for maintaining MEC survival *in vitro*.** Our laboratory has since focused on the signaling pathway that is initiated by GR activation was the model for uncovering anti-apoptotic signaling pathways in breast epithelial cells.

## Strategy

Because the activated GR is a potent transcriptional modulator we have used cDNA array analysis to identify genes that are directly turned on or down-regulated in MECs following GR activation. We hypothesize that these target genes may be upstream initiators of survival signaling pathways. Among the genes we have identified as direct GR transcriptional targets are several kinases and phosphatases (Figure 2). This finding provides the framework for a novel crosstalk between nuclear hormone receptor activity and kinase-mediated signaling cascades. For example, SGK-1 is a serine-threonine kinase of the AGC family that was originally identified in a rat mammary tumor cell line and prior to our investigations was primarily studied as a potential cell cycle regulatory protein. In kidney epithelial cells, SGK-1 appears to regulate epithelial sodium channel activity, although the exact mechanism of regulation is not understood.

**FIGURE 2** Model of GR-mediated cell survival




Activation of the GR signaling pathway via increased systemic glucocorticoids results in the immediate upregulation of several genes, including *Serum and Glucocorticoid-regulated Kinase (SGK)* and *MAP Kinase Phosphatase-1 (MKP-1)*. *SGK* and *MKP-1* encode a kinase and phosphatase, respectively, that in turn regulate the activity of key downstream transcription factors, thereby leading to a network of gene expression changes that favor tumor cell survival.

In 2001, we and others published that SGK-1 overexpression inhibits apoptosis in mammary epithelial cells and cerebellar neurons. Interestingly, the *C. elegans* homologue of SGK-1 was also recently found to be a critical component of insulin signaling, underscoring the fundamental importance of this kinase to cellular stress signaling.

The second pathway that we have linked to survival signaling in breast cancer cells is that of the MAPK-Phosphatase-1 (MKP-1)-induced inactivation of ERKs1, 2, and JNK. Although the MAPK pathway has traditionally been thought of as a proliferative pathway, we have recently shown that in the acute setting of stress to the cells (e.g., growth factor withdrawal or chemotherapy treatment) MAPK activation is required for efficient apoptosis. Thus, signals that lead to a sudden inactivation of MAPK signaling, such as activation of MKP-1, are predicted to result in the inhibition of cell death.

**We are using both high throughput functional genomics and traditional molecular biology approaches to address the mechanism whereby GR activation prevents apoptosis in breast cancer cells.** This pathway serves as an excellent model for uncovering anti-apoptotic signaling pathways that contribute to breast cancer, and dovetails nicely with the rich experimental history of the inquiry into the molecular pathways of steroid hormone action.

Having begun to identify some of the pathways downstream from GR activation in mammary epithelial cells, we next asked the question whether glucocorticoids might inhibit breast cancer cell apoptosis in a xenograft model of breast cancer. Using MDA-MB-231 cells that are ER-, PR-, Her2Neu-, and GR positive, we showed that administration of glucocorticoid inhibited apoptosis in response to chemotherapy. These findings raised the possibility that altered cortisol regulation may affect mammary cancer biology. In order to study altered cortisol in a model that might have translational relevance, we initiated a collaboration with Dr. Martha McClintock, a biopsychologist at the University of Chicago's Institute of Mind and Biology.

*These findings raised the possibility that altered cortisol regulation may affect mammary cancer biology.*

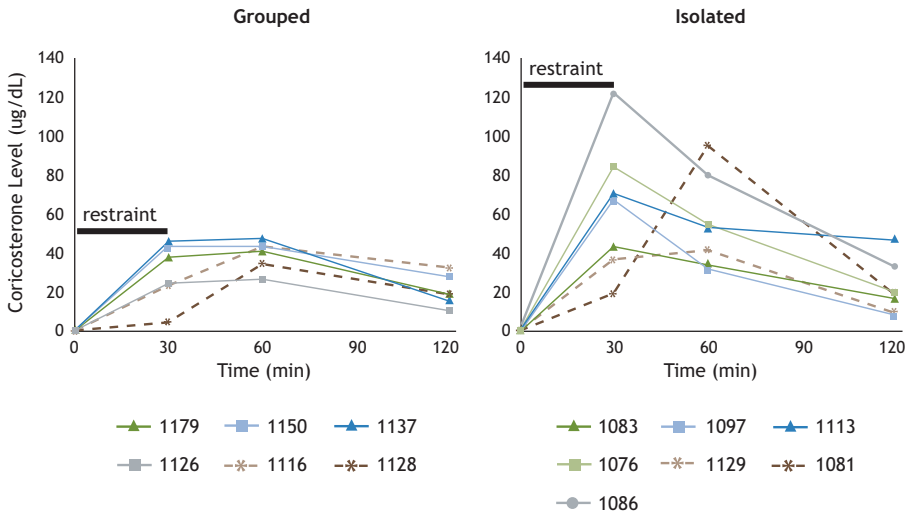
Dr. McClintock had observed that rats that are placed in social isolation develop mammary tumors earlier than a control group of group-housed rats. Isolated rats also appear more "vigilant" and anxious as measured by standard behavioral tests. Dr. McClintock's initial studies determined that the more "vigilant" behavior correlates with deregulation of the corticosterone response to a restraint stressor.

Our laboratory has now begun to study mammary gland tumors in a transgenic SV40 Tag model (obtained from the NCI's Mouse Models of Human Cancer Consortium) where human breast cancer is recapitulated both in terms of natural history and pathologic characteristics.

We obtained a CPHHD (P50) grant from the NIH that has allowed us to study both rat and mouse transgenic models of mammary cancer as they relate to the physiologic changes seen with social isolation. Our data demonstrate the SV40-Tag mice subjected to chronic social isolation exhibit a much brisker corticosterone response to a thirty minute restraint test.

This finding suggests that chronic social isolation alters the physiological response to an acute stressor. We hypothesized that repeated mild daily stressors may therefore cause an increased glucocorticoid response

**FIGURE 3** Normalized serum corticosterone levels (Ug/dL) following a 30-minute restraint performed on grouped (n=6) or isolated (n=7) SV40-Tag mice

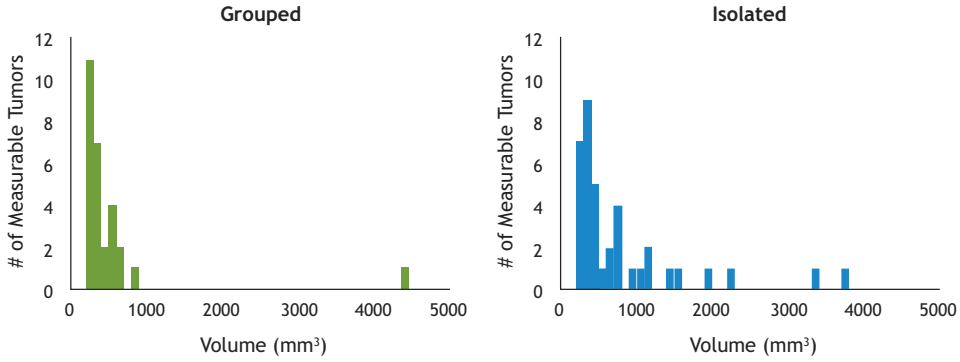



in target organs, including the mammary gland, leading to decreased apoptosis and larger overall tumor growth in isolated animals. Experiments are ongoing to examine gene expression in these tumors.

To our knowledge, **this is the first time that the role of a social environment has been studied in detail with respect to its effect on corticosterone responses and cancer biology in a well-defined animal model** (Figures 3 and 4).

By examining the activation of tumor cell signaling pathways, apoptotic indices, and tumor growth rates following long-term social isolation, the role of changes in the physiologic stress response will be determined.

**FIGURE 4** Measurable tumor volumes (>200mm$^3$) of grouped versus isolated mice differ at 22 weeks of age




Tumor volumes calculated using the standard formula: a x b2 x 0.52, where "a" is the longest and "b" is the shortest diameter. An ANOVA test was performed using log-transformed volumes to determine the significance of the difference ($p<0.05$).

## Acknowledgements

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## Suzanne D. Conzen, MD

Dr. Conzen received her B.A. from Brown University and her M.D. from the Yale School of Medicine, as well as an M.Sc. from the University of London's School of Hygiene and Tropical Medicine. She then completed clinical training in Internal Medicine (Cornell), and Hematology/Oncology (Dartmouth), followed by a molecular oncology fellowship at Dartmouth Medical School (as a Howard Hughes Medical Institute Fellow). In addition she did postdoctoral laboratory research at the University of Chicago's Ben May Institute for Cancer Research.

Dr. Conzen is currently an Associate Professor of Medicine at the University of Chicago, with secondary appointments in the Cancer Center, the Ben May Department for Cancer Research, the Committee on Molecular Medicine, and the Institute of Mind and Biology. She is co-director of the University of Chicago Cancer Center's Program in Cell Signaling and Gene Regulation, and serves as Chair of the Cancer Etiology Study Section of the NIH.

Dr. Conzen's laboratory and clinical focus are on identifying molecular pathways in breast cancer as novel targets for effective prevention and treatment. For example, her laboratory has identified an important role for glucocorticoid receptor signaling in breast cancer cell survival under conditions of physiological stress. This discovery led her to participate in the NIH-funded CPHHD, University of Chicago Center for Interdisciplinary Health Disparities Research, where she collaborates with social scientists and biopsychologists to study how the social environment and the stress response can alter mammary gland cancer biology in a transgenic model of human breast cancer.

# Bioinformatic Analyses of Gene-Social Environment Interactions in Cancer

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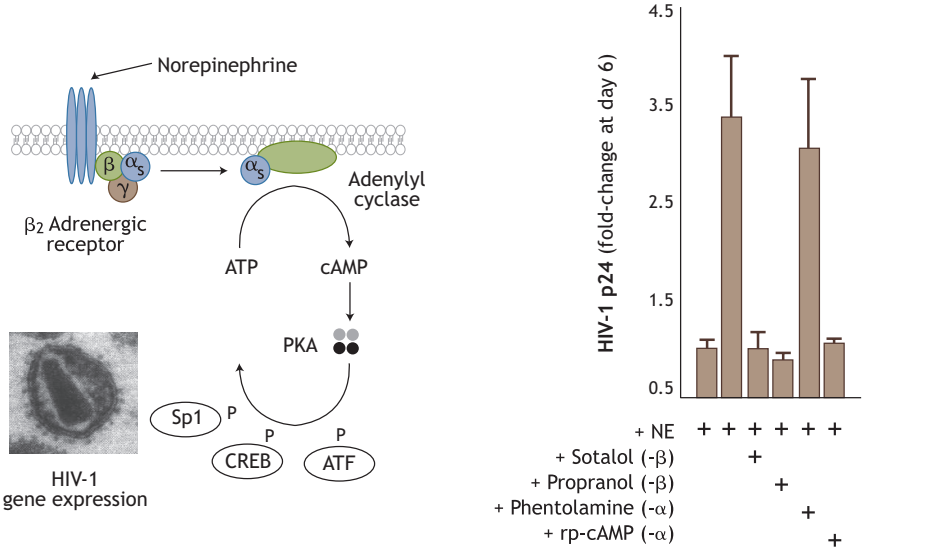
Characteristics of the social environment are known to affect disease risk,[1,2] but the biological mechanisms mediating these effects remain poorly understood. Our research program seeks to identify the "social signal transduction" pathways that mediate the effects of external social factors on the internal molecular biology of disease in the context of viral infections and cancer. Our initial work with viral infections has highlighted a critical role for the autonomic nervous system in structuring peripheral gene expression profiles via the effects of sympathetic nervous system (SNS) neurotransmitters on cellular β-adrenergic signaling pathways. More recent work has shown that β-adrenergic signaling can also influence the biology of liquid and solid tumor cells. We are now applying new genomics-based bioinformatics strategies to translate these findings into the clinical setting, and to identify additional biological signal transduction pathways that mediate biobehavioral influences on tumor cell biology. Our ultimate objective is to understand biobehavioral influences on cancer from a molecular biology perspective, and develop rationally targeted protective interventions to mitigate those effects.

## Viral Pathogenesis

Infectious diseases have long been known to be sensitive to stress, and our early studies sought to understand the molecular mechanism of these effects in the context of HIV-1 infection. Initial epidemiologic studies documented accelerated disease progression in stressed individuals,[3–5] and subsequent clinical studies identified variations in SNS activity as a potential biological mediator of those effects.[6,7] In vitro analyses of the molecular biology of HIV-1 replication showed that the primary SNS neuroeffector molecule norepinephrine (NE) could accelerate viral replication in activated T lymphocytes by increasing viral co-receptor expression,[6, 8] activating cellular transcription factors that drive viral gene expression,[6] and impairing cellular antiviral responses mediated by Type I interferons and other cytokines.[9, 10] These effects were mediated by NE ligation of leukocyte β-adrenoreceptors, and subsequent activation of the cellular cyclic 3'-5' adenosine monophosphate (cAMP)/ Protein Kinase A (PKA) signaling pathway (Figure 1, p. 28).[9]

*This line of research represents the first instance in which researchers have been able to definitively identify the mediating molecular mechanisms of a social risk factor for any somatic disease.*

**FIGURE 1** Regulation of viral gene expression by the host cell β-adrenergic signaling pathway




Pharmacologic antagonists of β-adrenoreceptors and the cAMP/PKA signaling pathway inhibit HIV-1 replication in activated T lymphocytes.

Subsequent *in vivo* studies in the rhesus macaque model of Simian Immunodeficiency Virus confirmed that social stress could suppress Type I interferon responses and increase viral replication in lymph nodes from experimentally infected animals.[11] Viral gene expression was specifically enhanced in proximity to SNS neural fibers within lymph nodes, and experimental social stress significantly increased the density of lymph node SNS fibers, leading to an increased frequency of viral replication (Figure 2, p. 29).

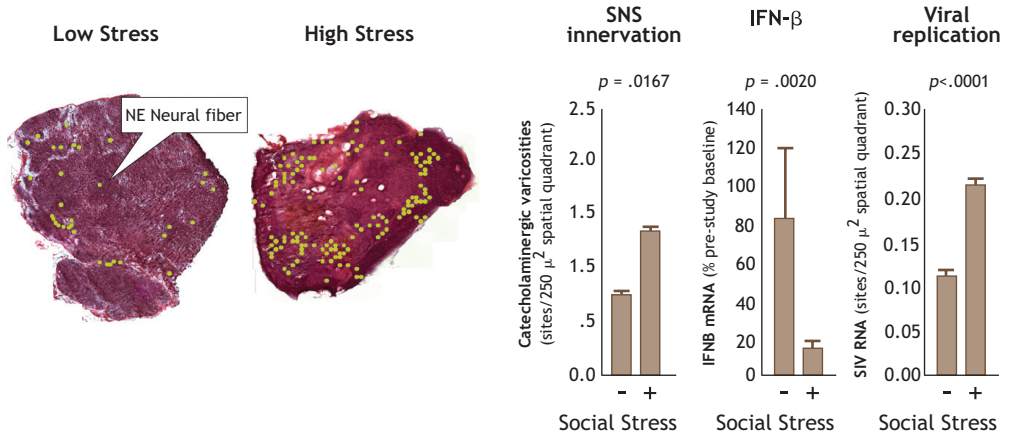
These results suggest an unexpected stress-induced plasticity in peripheral sympathetic neurons with the capacity to impact local molecular pathogenesis. This line of research represents the first instance in which researchers have been able to definitively identify the mediating molecular mechanisms of a social risk factor for any somatic disease. **These studies also suggest that SNS-induced activation of the cellular cAMP/PKA signaling pathway can play a key role in mediating biobehavioral influences on the biology of infectious disease.**

## Cancer-related Viruses

To determine whether SNS signaling might exert similar effects on the activity of a tumor-associated virus, we have analyzed the β-adrenregic regulation of gene expression by the Kaposi's Sarcoma-associated Herpes Virus (KSHV/Human Herpesvirus 8). KSHV induces aberrant growth of vascular endothelial cells in Kaposi's Sarcoma, and B cell proliferation in Primary Effusion Lymphoma (PEL) and Multicentric Castleman's Disease. Key to its pathogenic potential is the ability of KSHV to enter a protracted transcriptional latency in which it evades immune clearance, and then resumes "lytic replication" in response to unknown physiologic signals. Our studies identified the SNS neurotransmitter NE as a key physiologic trigger for KSHV reactivation and expression of its malignancy-inducing gene products (Figure 3, p. 29).[12]

Subsequent analyses mapped two molecular mechanisms for these effects that are both triggered by NE ligation of β-adrenergic receptors on latently

**FIGURE 2** Sympathetic nervous system regulation of viral replication in primate lymph nodes



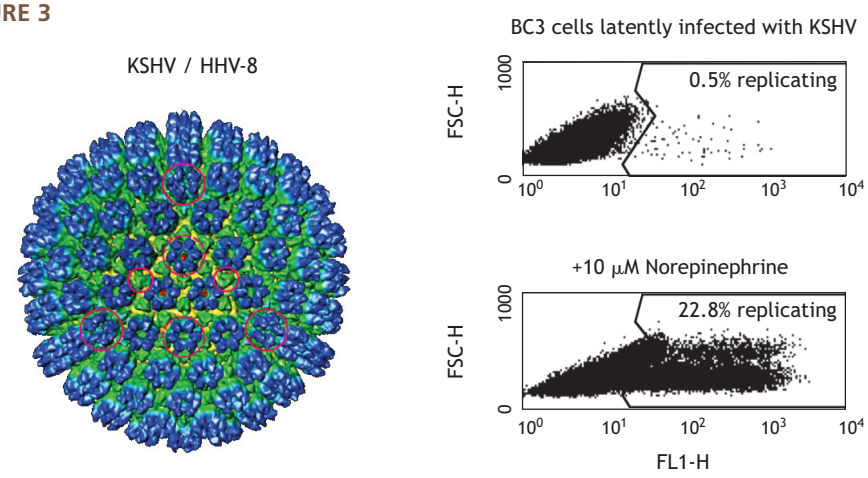

Lymph nodes biopsied from rhesus macaques subject to periodic social hierarchy disruption show increased density of sympathetic neural fibers, decreased interferon-β response to Simian Immunodeficiency Virus, and increased viral replication.

infected B lymphocytes:

1. Down-stream activation of cellular CREB/ATF and Sp1 transcription factors induces expression of the key viral transcription factor ORF50/*RTA*, and
2. PKA phosphorylation of ORF50/*RTA* to enhance its trans-activating capacity.

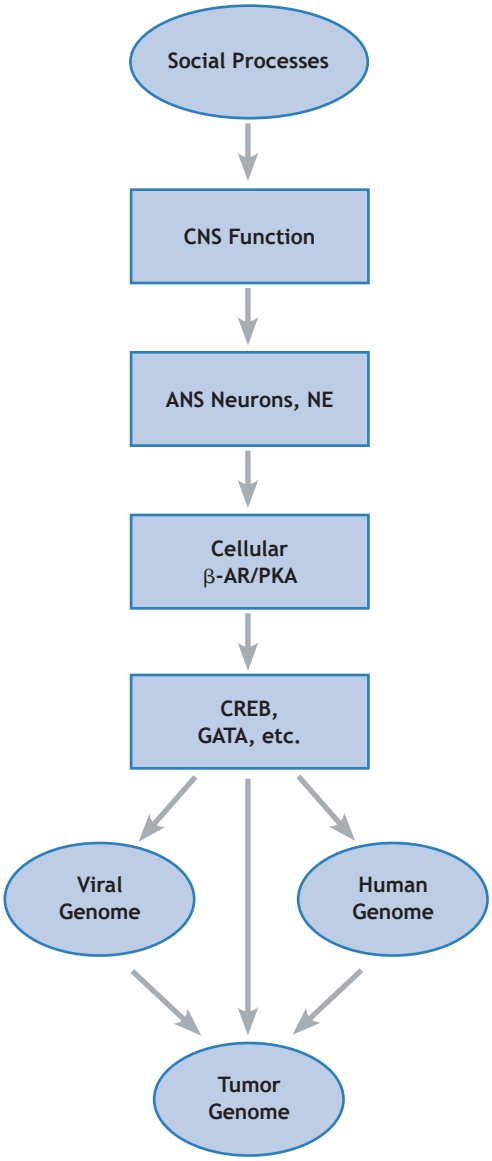
**In addition to defining the physiologic signaling pathways that mediate stress-induced activation of KSHV, these studies have identified a novel therapeutic approach using β-adrenergic agonists to flush latent KSHV into active lytic replication for eradication by replication-dependent nucleoside analogue drugs (e.g., ganciclovir).**

**FIGURE 3**




The sympathetic nervous system neurotransmitter norepinephrine reactivates lytic replication and expression of oncogenic genes in Primary Effusion Lymphoma cells latently infected with Human Herpesvirus 8 (Kaposi's Sarcoma-associated Herpesvirus).

**FIGURE 4** "Social signal transduction" pathway for regulation of cancer cell gene expression




Social factors influence central nervous system control of peripheral sympathetic neurons, resulting in norepinephrine release and subsequent activation of the cellular β-adrenergic/cAMP/PKA signaling pathway. PKA activation of cellular transcription factors can modulate gene expression by tumor cells, elements of the tumor microenvironment, and cancer-associated viruses.

## Solid Tumors

Given the key role of β-adrenergic signaling in regulating viral gene expression, we next sought to determine whether this pathway might also impact gene expression by solid tumor cells. In collaboration with Susan Lutgendorf[1] (University of Iowa) and Anil Sood[2] (University of Texas, M.D. Anderson Cancer Center), our studies of human ovarian carcinoma cells *in vitro* and in orthotopic mouse models show that the SNS neuroeffector molecule NE can up-regulate several genes instrumental in tumor cell invasion and metastasis (e.g., *VEGF*, *IL6*, *IL8*, and *MMP9*).[13-15] My laboratory has focused on defining the intracellular signal transduction pathways that mediate NE induction of target gene promoters. Beta-adrenergic signaling through the cAMP/PKA and Src signaling pathways plays a key role (Figure 4), suggesting a potential impact of SNS activity on tumor cell biology *in vivo*.

## Bioinformatics for Gene-Environment Interactions in Cancer

To assess the role of β-adrenergic signaling in the molecular biology of clinical cancer, we have developed several new bioinformatics strategies for analyzing DNA microarray data in terms of the up-stream regulatory forces that shape broad patterns of gene expression (e.g., the activity of transcription factors, epigenetic process, and genomic damage).

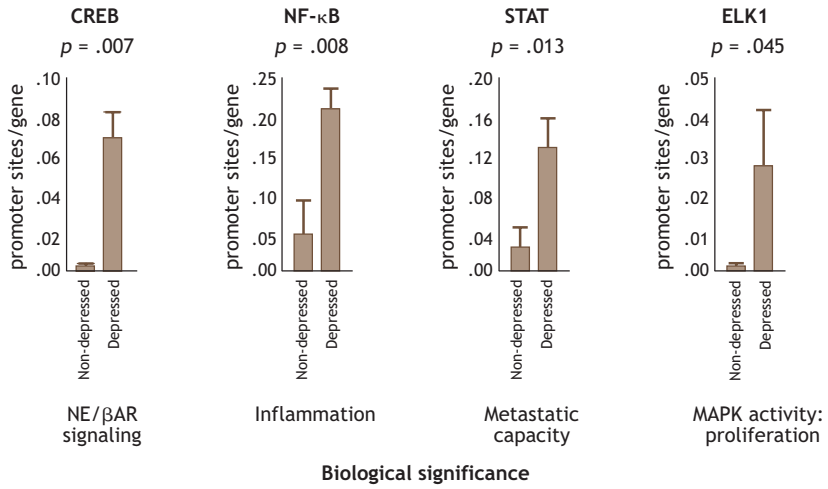
These tools are now playing a central role in our clinical studies of cAMP/PKA signaling *in vivo*, and in efforts to identify additional biological signaling pathways that mediate biobehavioral influences on cancer biology. Examples include:

1. The TELiS search engine (www.telis.ucla.edu) uses data on the prevalence of transcription factor-binding motifs in the

[1] Dr. Lutgendorf's research has been supported by the National Cancer Institute (R21 CA88293 and R01 CA104825 "Biobehavioral-Cytokine Interactions in Ovarian Cancer").

[2] Dr. Sood's research has been supported in part by the National Cancer Institute (R01 CA110793 "Tumor metastasis: Biobehavioral mechanisms" and R01 CA109298 "Ovarian Cancer: Mechanisms of Neuroendocrine Regulation").

**FIGURE 5** Bioinformatic analysis of transcription control pathways activated in ovarian carcinomas from patients with biobehavioral risk profiles (high depressive symptoms and low social support)

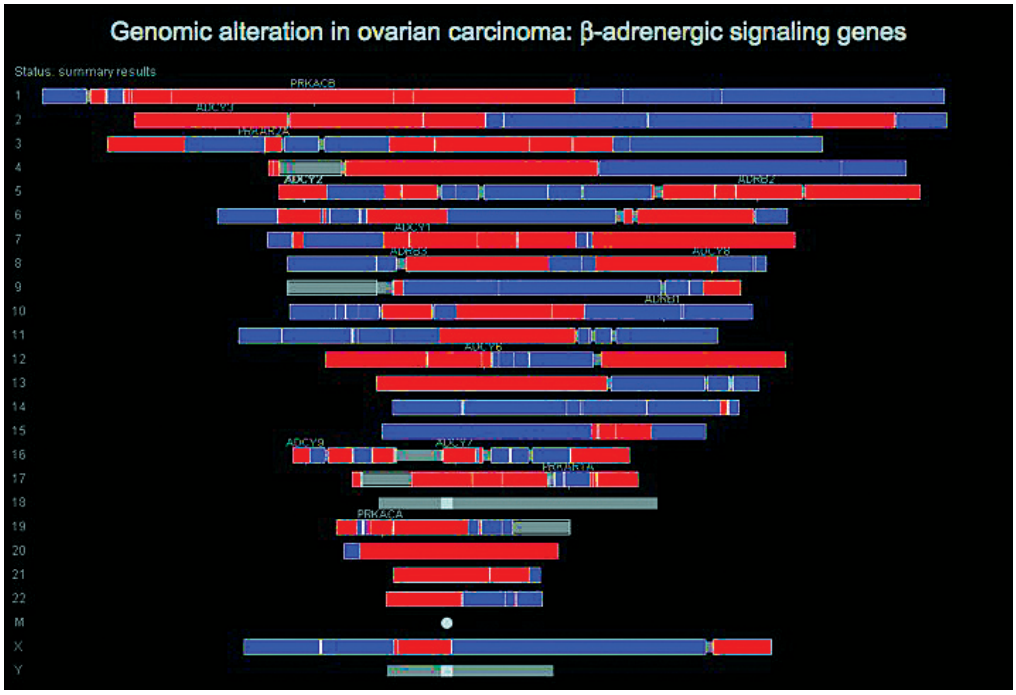



promoters of differentially expressed genes to identify transcription control pathways that are activated in association with a measured phenotype.[16] This approach has been employed in studies of primary ovarian cancer cells to identify increased activity of CREB/ATF, NF-$_k$B/Rel, STAT, and ETS transcription factors in tumors from individuals with adverse behavioral risk profiles (high levels of depression and low social support; Figure 5). Additional studies have identified biological mechanisms by which other behavioral influences such as sleep/circadian disruption might influence inflammatory biology.[17] This bioinformatics-based analysis provides an unbiased and global strategy for identifying candidate transcription control pathways that mediate biobehavioral influences on tumor cell biology *in vitro*, *in vivo*, and in clinical patient samples.

2. SpAnGEL analysis of chromosomal structural alterations[18,19] has been used to relate patterns of genomic alteration to neuroendocrine signaling pathways in primary ovarian cancers. Genomic alterations play a key role in solid tumor pathogenesis by amplifying genes that support cell proliferation and metastasis, and deleting growth regulators (e.g., tumor suppressors). Bioinformatic analyses of genomic alterations in chromosomal regions containing adrenergic signaling genes (β adrenorceptors, adenylyl cyclases, PKA subunits) provide statistical may be under selective pressure for increased activity of the β-adreno-receptor/PKA signaling pathway that mediates cellular response to NE within the tumor microenvironment.

   The observed pattern of amplifications on chromosomes 1p, 2p, 3p, 5p, 5q, 7p, 8p, 8q, 12p, 16p, and 19p (containing adrenergic signaling genes) and deletion of 3p and 17q (containing adrenergic regulatory genes) is independent of other known growth pathways and highly improbable by chance alone (p = .00006; Figure 6, p. 32). These results provide a novel indication that the neuroendocrine system might help shape the evolution of tumor genomes.

**FIGURE 6** β-AR pathway genes: ovarian carcinoma

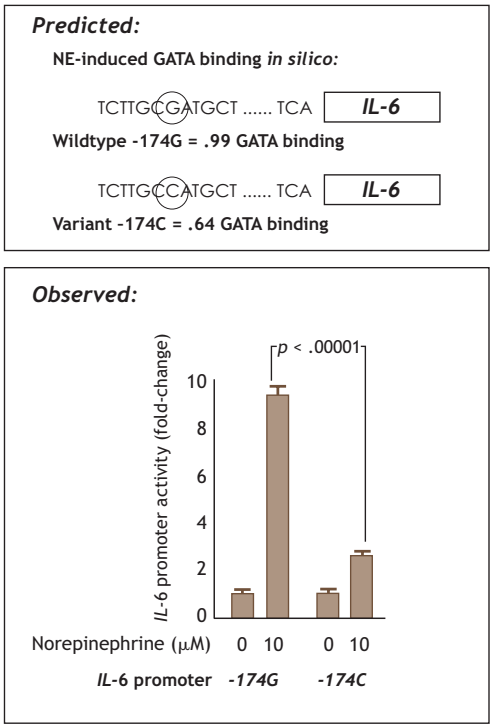



Relationship between regional genomic alterations and β-adrenergic signaling genes in ovarian cancer. Red indicates chromosomal regions of increased gene expression (genomic amplification or epigenetic de-repression) and blue indicates chromosomal regions of decreased gene expression, averaged over 10 cases.

3. The PromoterSNP database provides a bioinformatic platform for predicting gene-environment interactions that might alter cellular transcriptional response to environmental risk factors for cancer onset or progression. All known human single nucleotide polymorphisms (SNPs) have been analyzed computationally for their potential ability to modify interactions between environmentally responsive transcription factors and their response elements in the promoters of human genes. For example, chronic inflammation is believed to contribute to several types of cancer, and PromoterSNP analyses have predicted that a common SNP in the promoter of the pro-inflammatory *IL-6* gene (–174 G/C) might render individuals carrying the C allele relatively resistant to NE-induced up-regulation of *IL-6* transcription.

*In vitro* analyses of the two promoter variants confirm the bioinformatic prediction that the -174G allele is substantially more responsive to the SNS neuroeffector NE, suggesting that carriers of this allele may be more vulnerable to stress-induced *IL-6* up-regulation and consequent risk of neoplastic disease (Figure 7, p. 33). Such results may explain the mixed association between the *IL-6* –174G allele and cancer progression.[20–22] Adverse effects may require an environmental challenge to exert their biological impact on inflammatory gene expression. This candidate and more than 10,000 other SNPs predicted to modulate transcriptional response to SNS activity are now ready for empirical confirmation in molecular epidemiology studies that simultaneously assess environmental risk factors and host genetic characteristics.

**FIGURE 7** Bioinformatic prediction of gene-environment interaction in expression of human *IL-6*




Over-expression of this proinflammatory cytokine is a risk factor for multiple tumor types, and bioinformatic analyses using the PromoterSNP algorithm predict that a known single nucleotide polymorphism in the *IL-6* promoter (–174 G/C) will modulate transcriptional response to norepinephrine activation of GATA transcription factors. Biological reporter assays confirm that prediction, with the –174G allele showing increased transcriptional response to norepinephrine in HeyA8 ovarian carcinoma cells.

This in silico analysis provides a hypothesis-driven strategy for identifying genetic polymorphisms that modulate individual vulnerability to socio-environmental risk factors (or any other environmental exposure with a known transcriptional mediator).

## Future Directions

These three examples show how bioinformatic approaches rooted in the molecular mechanisms of gene regulation can be harnessed to understand biobehavioral influences on tumor biology at the level of global genomic activity. Our challenge now is to translate these genomics-based hypotheses into clinically effective interventions that can protect patients with biobehavioral risk factors against the adverse effects of stress biology (e.g., β-adrenergic/PKA signaling) on tumor cell biology.

Pharmacologic intervention at the level of physiologic signal transduction represents a promising approach. Beta blockers have efficiently abrogated NE effects on tumor biology and viral gene expression in model systems,[9,10,12,14] and these agents are appealing candidates for clinical proof-of-concept studies due to their favorable safety profiles and low cost. Serotonin receptor agonists are also known to inhibit cAMP/PKA signaling,[23] and could provide an alternative pharmacologic approach. The tumor gene expression fingerprints associated with biobehavioral risk factors provide a set of molecular biomarkers that can be monitored to assess the biological impact of these interventions before changes in clinical disease are evident.

*Our challenge now is to translate these genomics-based hypotheses into clinically effective interventions that can protect patients with biobehavioral risk factors against the adverse effects of stress biology on tumor cell biology.*

In addition to these translational opportunities, several basic science studies are underway to define more fully the genetic basis for socio-environmental regulation of gene expression in cancer. These include

molecular epidemiologic studies assessing the role of genetic polymorphisms in modulating vulnerability to socio-environmental influences, and empirical analyses of tumor genome dynamics to more fully define the effects of local neuroendocrine signaling in shaping the molecular evolution of tumors. The observation that experimental social stress can increase neural density and viral disease pathogenesis in lymphoid organs raises the possibility that similar dynamics might occur in tumor structures or their local microenvironment. Finally, with the exception of the well-explored virus-associated cancers and ovarian epithelial tumors, little is known about the extent to which other prevalent solid tumors are subject to regulation by the neuroendocrine system. Epidemiologic evidence of social risk factors for breast and prostate cancer progression[24–26] suggests that neuroendocrine regulation may be relevant to a broad spectrum of human cancers.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- National Cancer Institute (R01 CA116778 *Combinatorial genomics in cancer*, R01 CA110793 *Tumor metastasis: Biobehavioral mechanisms*, R01 CA109298 *Ovarian cancer: Mechanisms of neuroendocrine regulation*)
- National Institute of Allergy and Infectious Diseases (R01 AI52737 *Autonomic nervous system regulation of HIV-1 replication*), the National Institute of Mental Health (R01 MH049033 *Simian AIDS: Social stress, endocrine, and immune function*)
- National Institute of Dental and Craniofacial Research (R01 DE015790 *Genomics and proteomics of oral precancer progression*, R03 DE106569 *Expression-based identification of OHNC genomic changes*)
- National Institute of Research Resources (P20 RR020645 *Integrative biology of brain, inflammation, and asthma*)
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- HopeLab Foundation
- John D. and Catherine T. MacArthur Foundation
- James B. Pendelton Charitable Trust

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## Steven W. Cole, PhD

Dr. Steven Cole's laboratory studies how stress hormones regulate the activity of human and viral genomes. His research seeks to determine how stress suppresses cellular immune responses, enhances activity of HIV-1 and cancer-causing viruses such as Human Herpesvirus 8, and regulates the function of solid tumor cells (e.g., modulating invasion and metastasis of ovarian carcinoma). To accelerate discovery and translation of these findings, Dr. Cole's laboratory also develops new mathematical tools for analyzing complex gene networks.

Dr. Cole's research focuses on the role of cAMP/PKA signaling in regulating global patterns of gene expression. A broad array of viral genomes respond to this cellular signaling pathway, and Cole's lab has mapped several points of interaction between PKA signaling and viral replication cycles. In the case of HIV-1, PKA up-regulates the co-receptors CCR5 and CXCR4, induces cellular transcription factors to interact with the viral Long Terminal Repeat promoter, and suppresses transcription of antiviral Type I interferons. In the case of the Kaposi's Sarcoma Herpes Virus (KSHV/HHV-8), PKA up-regulates transcription of the key viral transcription factor RTA and post-translationally modifies its transcription-activating capacity. Similar mechanisms have been identified for HCMV and HSV-1 and -2. Cole and his colleagues are now evaluating pharmacologic kinase modulators and gene therapeutic manipulation of PKA and downstream transcription factors. These principles have already been applied to developing novel vector-born molecular adjuvants to enhance vaccine-induced cellular immune responses against viruses and tumors.

Dr. Cole is an Associate Professor of Medicine in the Division of Hematology-Oncology at UCLA. After receiving his Ph.D. in Biological Psychology from Stanford University in 1992, he completed an NIH Postdoctoral Fellowship in Health Psychology at UCLA and a second Postdoctoral Fellowship in molecular virology in the Norman Cousins Program at UCLA. He joined the UCLA Medical School Faculty in 1997, and holds a joint appointment in the Department of Psychiatry and Biobehavioral Sciences. Dr. Cole is a member of the Jonsson Comprehensive Cancer Center (Signal Transduction and Cancer Virology programs), the Norman Cousins Center for Psychoneuroimmunology, the UCLA AIDS Institute, and the UCLA Molecular Biology Institute. He has received a variety of teaching and scientific awards, including Stanford University's Centennial Teaching Award and the Cornellius L. Hoppers Award from the University of California's University-wide AIDS Research Program. Dr. Cole has also served as a scientific consultant to the National Cancer Institute and the Institute of Medicine at the National Academy of Sciences, and as a scientific fellow and instructor at the Santa Fe Institute for Complex Systems. He also serves as Vice President for Research at HopeLab – a nonprofit developer of social technology solutions for cancer prevention and control.

Dr. Cole serves on the editorial board of several scientific journals, on the scientific council of the Norman Cousins Center and the PNI Research Society, and is a grant reviewer for the NIH Center for Scientific Review in the area of biobehavioral influences on disease. He also serves as a consultant on statistical modeling and research design for a variety of government and private sector agencies, including the United States Navy and Air Force, the World Health Organization, Stanford University's Graduate School of Business, the Anderson Graduate School of Business at UCLA, the University of Chicago School of Business, Citibank Corporation, and the Terman Study of the Gifted.

Dr. Cole is a frequent presenter at research meetings in the area of biobehavioral influences on disease, and the author of more than 50 peer-reviewed scientific publications. His research is funded by grants from the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, the National Institute of Aging, the MacArthur Foundation, and the James Pendelton Charitable Trust. He is a member of the American Association for the Advancement of Science, the Psychoneuroimmunology Research Society, and the American Statistical Association.

PERSPECTIVES FROM A PNI PARADIGM

# Stress-and Depression-Associated Dysregulation of Cytokine Production and Immune Function: Health Implications

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The field of psychoneuroimmunology (PNI) focuses on the interactions among the central nervous system (CNS), the endocrine system, and the immune system, and the impact of these interactions on health. Modulation of the immune response by the CNS is mediated by a complex network of signals functioning via bi-directional communication among the nervous, endocrine, and immune systems. The hypothalamic-pituitary-adrenal (HPA) and the sympathetic-adrenal-medullary (SAM) axes are two pathways by which immune dysregulation is produced. The interactions among these systems are modulated by biological mediators that interact with and affect cellular components of the immune system.

Both major and minor stressful events can have direct adverse effects on a variety of immunological mechanisms; animal and human studies have provided convincing evidence that these immune alterations are consequential for health. Several studies from our laboratory and others support this conclusion. For example, to help demonstrate causal relations between psychosocial stressors and the development of infectious illness, investigators have inoculated subjects with several different types of vaccines.[1–6]

In one early study from our laboratory, stress and social support were related to the virus-specific antibody and T-cell responses to a hepatitis B vaccine among medical students taking exams.[1–6] In addition, the chronic stress associated with caregiving for a spouse with Alzheimer's Disease (AD) was associated with poorer antibody and T-cell responses to an influenza virus vaccine, and a poorer antibody response to a pneumococcus vaccine compared to well-matched control subjects.[2,3]

Vaccine responses demonstrate clinically relevant alterations in an immunological response to challenge under well-controlled conditions. Accordingly, they act as a proxy for responses to an infectious agent. Therefore, in individuals who produced delayed, weaker, and shorter-lived immune responses to vaccines, it is reasonable to assume these same individuals would also be slower to develop immune responses to other pathogens and perhaps to tumor-associated antigens on tumor cells of immunogenic tumors.

Consistent with this argument, adults who show poorer responses to vaccines also experience higher rates of clinical illness, as well as longer-lasting infectious episodes.[7,8] In further support of this idea, Cohen et al. showed that human volunteers who were inoculated with five

different strains of respiratory viruses showed a dose-dependent relationship between stress and clinical symptoms observed after infection.[9] Taken together, these studies show that psychological stress can influence immune function, alter the pathophysiology of infection, and have consequences for health.

*We now have evidence to suggest that stress hormones may also affect skin cancer progression by directly affecting the expression of proangiogenic factors by tumor cells.*

Experiments using animal models have provided the tools to conduct a comprehensive analysis of neuroendocrine-immune interactions under a variety of experimental conditions. Furthermore, such studies have supported the data obtained from human subjects and have also provided important information on mechanisms, including the effects of various stressors on the pathophysiology of infectious agents administered at different anatomical sites.

Within this experimental paradigm, stress diminishes vaccine responses, exacerbates viral and bacterial pathogenesis, slows wound healing, and alters autoimmune diseases.[10–14] These studies have demonstrated that **stress hormones inhibit the trafficking of neutrophils, macrophages, antigen presenting cells, NK cells, and T and B lymphocytes; suppress the production of proinflammatory cytokines and chemokines; down-regulate the production of cytokines necessary for the generation of adaptive immune responses; and impair effector functions of macrophages, NK cells, and lymphocytes.**

As already mentioned, a series of studies have demonstrated that psychological stress-induced immune dysregulation can play an important role in the early phases of wound healing.[15–17] The data suggest that psychological stress can affect the local wound environment in skin through the modulation of proinflammatory cytokine production.[16] In addition to showing that stress can slow the healing of small surface skin wounds, these studies may have relevance for surgery. Broadbent and colleagues examined the effect of pre-operative stress and worry on wound healing following surgery for inguinal hernia. Greater pre-operative perceived stress was negatively correlated with levels of *IL-1* in the wound fluid, and greater worry about the operation was associated with lower matrix metalloproteinase-9 (MMP) levels in the wound fluid as well. Patients who expressed more worry experienced a more painful, poorer, and slower recovery following the surgery. This study suggests that psychological stress associated with surgery impaired the inflammatory response and impacted the wound healing process.[18] Consistent with this report, in three separate studies, we found that stress-induced down-regulation was associated with a delay in wound healing, and that the delay was negatively correlated with *IL-1*β expression in PBLs.[15–17] Data from wound studies using restraint stressed mice are similar to the data obtained in the studies with human subjects.[11]

The modulation of the wound healing process by stress and depression has direct implications for cancer since many of the immune processes involved in wound healing are also part of the process of tumor progression. Examples of this include altered production of proinflammatory cytokines, as well as cell growth and angiogenesis.[19–22]

Both the literature on humans and the research from animal models support the hypothesis that stress-induced immune

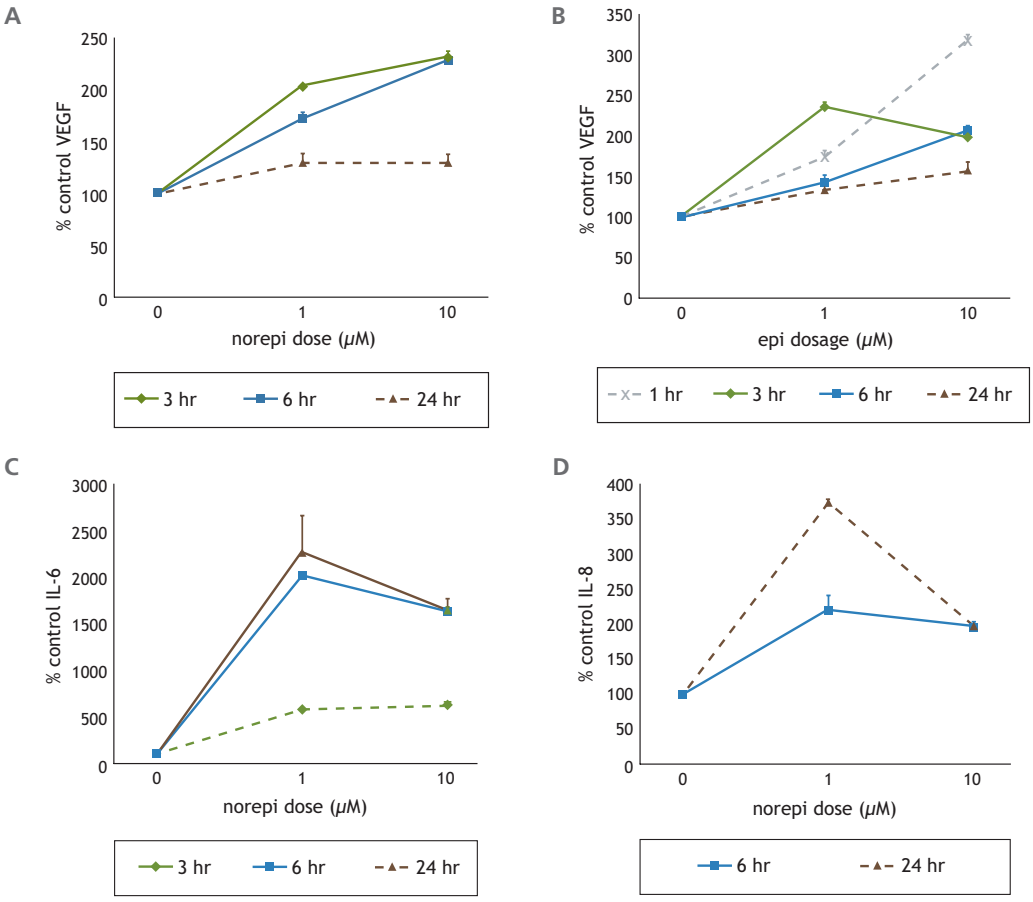
dysregulation produces biologically significant changes in various aspects of the cellular immune response with clear health consequences. Importantly, many of these outcomes are a direct result of changes in T-cell function.[23]

### *In Vitro* Studies

*In vitro* studies show that catecholamine hormones, epinephrine and norepinephrine, may play a role in the progress of immunogenic tumors. Besides its immune-related effects described above, recent studies suggest that psychological stress can also have direct effects that may contribute to tumor progression.

Our work and that of others have shown that MMPs and vascular endothelial growth factor (VEGF) are factors that can be modulated by catecholamine hormones. For example, studies by Sood, Lutgendorf, and others have shown that norepinephrine (norepi) and epinephrine (epi) may influence the progression of ovarian cancer by modulating the expression of MMPs and the angiogenic cytokine, VEGF, in ovarian cancer cells. We now have evidence to suggest that stress hormones may also affect skin cancer progression by directly affecting the expression of proangiogenic factors by tumor cells.

FIGURE 1

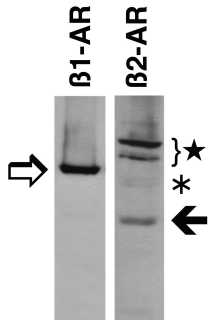



**(A)** and **(B)** VEGF, **(C)** *IL-6*, and **(D)** *IL-8* concentrations in culture supernatants from C8161 cells after treatment with norepi (A, C, and D) or epi (B). Levels of protein in culture supernatants were measured after treatment with 1, and 10 μmol/L norepi for 1, 3, 6, and 24 hours. Values are presented as percent change from untreated C8161 cells; Bars, SE.

We found that exposure of C8161 human melanoma cells to norepi resulted in greater secretion of factors implicated in melanoma tumor progression, i.e., VEGF, *IL-6*, and *IL-8* (Figure 1A, 1C, & 1D, p. 41). We also show that epi stimulates VEGF secretion (Figure 1B, p. 41). β1-adrenergic receptor (AR) and β2-AR were expressed in C8161 cells (Figure 2A). Evidence supporting the role of these receptors in the norepi-dependent effect is provided by our results showing that propranolol completely inhibited the norepi-dependent stimulation of VEGF release (Figure 2B). In addition, preliminary studies elucidating the downstream factors involved in the signaling pathway of the norepi-dependent modulation of VEGF expression suggests that the adenylate cyclase may not have a role but may involve the cAMP-dependent protein kinase (PKA) (Figure 3, p. 43).

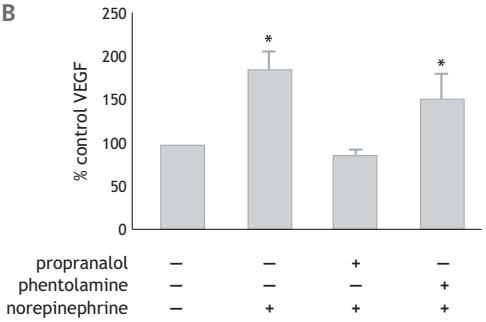
**FIGURE 2** Expression of β-ARs in C8161 cells

A




**(A)** Western blot analysis of β1-AR and β2-AR expression in C8161 melanoma cells. Lysates from C8161 cells probed for β1-AR revealed a band with an apparent molecular weight of 75 x $10^3$ $M_r$ (⇨). Several bands were observed when cell lysates were probed for β2-AR. A single band with molecular weight of about 47 x $10^3$ $M_r$ was expressed in C8161 cells (←) that is consistent with the unglycosylated protein. The band at about 65 x $10^3$ $M_r$ (✱) is the glycosylated receptor, while the bands at about 90 to 100 x $10^3$ $M_r$ result from dimerization (★). These bands were not observed in blots incubated with normal rabbit serum (not shown).

B




**(B)** Effect of β-blocker (propranolol) and α-blocker (phentolamine) on VEGF levels in the culture supernatants of C8161 cells in the presence or absence of norepi. Bars, SE. *P < 0.001, indicate differences from untreated control.

We observed the expression of β2-ARs on tumor cells in 18 out of 20 melanoma biopsies from primary (nodular, superficial spreading, and desmoplastic) and metastatic (lymph node and visceral metastasis) melanomas supporting the clinical relevance of our *in vitro* results (Table 1 and Figure 4, p. 43). That a majority of the melanoma tumors we examined showed the presence of β2-AR supports our hypothesis that melanoma tumor cells have the potential to respond to norepi and epi.

To our knowledge, this is the first evidence that demonstrates that stress-related activation of the SAM axis may have a role in melanoma tumor progression. The data suggest that norepi, a stress hormone produced after the activation of the SAM axis, may play a role in stimulating the aggressiveness of malignant melanoma.

## The Impact of Catecholamine Hormones on Angiogenesis and Tumor Progression

Our laboratory has been interested in the role that Epstein-Barr virus (EBV) plays in the development of nasopharyngeal carcinoma (NPC) for many years.[24] NPC is an EBV-associated malignant tumor with the highest incidence observed among Chinese from Hong Kong and southern China.[25] It is a highly invasive and metastatic head and neck cancer characterized by metastasis to the cervical lymph nodes and distant organs.[26] MMPs, the gelatinases MMP-2 and MMP-9 in particular, and VEGF have been implicated as contributing to the aggressiveness of highly metastatic NPC tumors.[27,28]

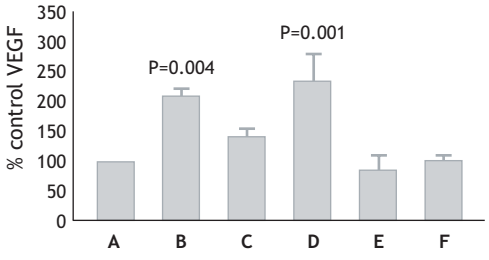
**TABLE 1** The presence of β2-AR in tumor cells within melanoma tumor biopsies

| | | |
|---|---|---|
| **PRIMARY MELANOMA** | **TYPE** | **POSITIVE/TOTAL** |
| | Nodular | 3/3 |
| | Superficial-spreading | 3/4 |
| | Desmoplastic | 3/3 |
| **METASTATIC MELANOMA** | **SITE** | |
| | Lymph node | 4/5 |
| | Visceral | 5/5 |

In previous studies, we described the establishment and characterization of an EBV DNA-positive NPC cell line (HONE-1) and an EBV DNA-negative NPC cell line (HNE-1).[29–31] In this study, we utilize these cells to examine the ability of norepi to up-regulate the expression of three factors that have roles in the progression of NPC tumors, i.e., MMP-2, MMP-9, and VEGF. It is known that the ability of norepi to up-regulate MMP-2, MMP-9, and VEGF is mediated by β-ARs.

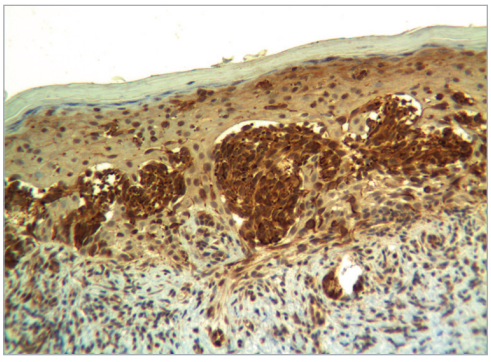
**We found that norepi can up-regulate the production of MMP-2, MMP-9, and VEGF by HONE-1 cells** (Figure 5, p. 44). **Furthermore, we show that norepi** (Figure 6A, p. 44) **and epi** (Figure 6B, p. 44) **can stimulate the invasive capability of HONE-1 cells through the expression of MMPs** (Figure 6C, p. 44). We also show that norepi-treated NPC cells exhibit up-regulated release of biologically active VEGF (Figure 7, p. 45). Experiments performed to determine if NPC tumor cells express β-ARs showed that NPC

**FIGURE 3**



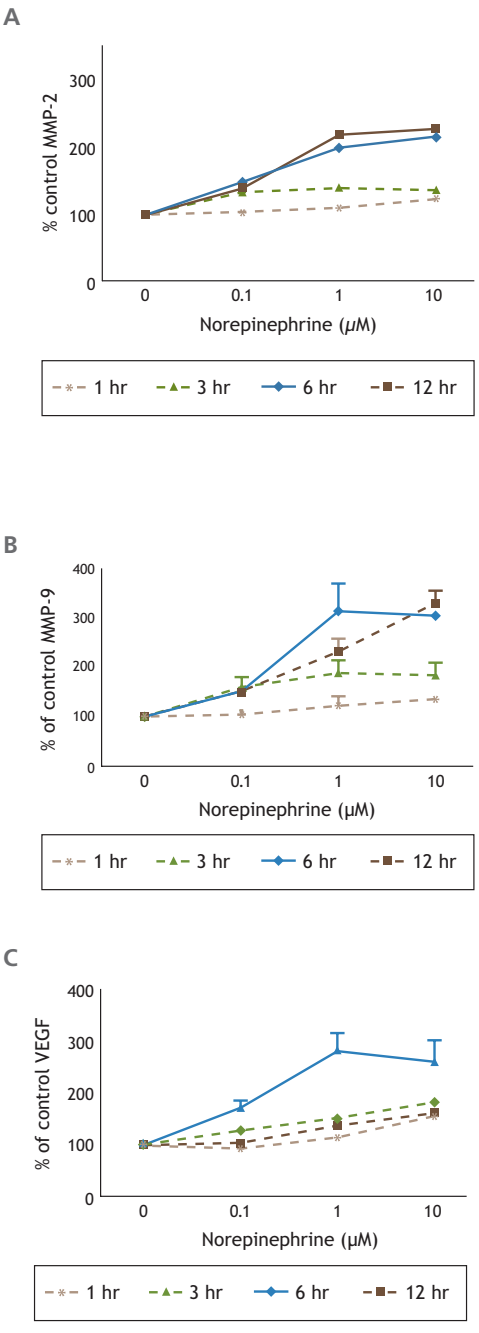

**(A)** C8161 cells were grown for 3 hours in serum-free media alone, **(B)** 1 μmol/L norepi, **(C)** 10 μmol/L forskolin, **(D)** 10 μmol/L isoproterenol, **(E)** 100 μmol/L 8-CPT, or **(F)** 100 μmol/L 6-Bnz-cAMP. VEGF protein concentrations in culture supernatants were assessed by ELISA. Values are presented as percent change from untreated C8161 cells. Bars, SE. P values indicate difference from untreated control levels.

**FIGURE 4**



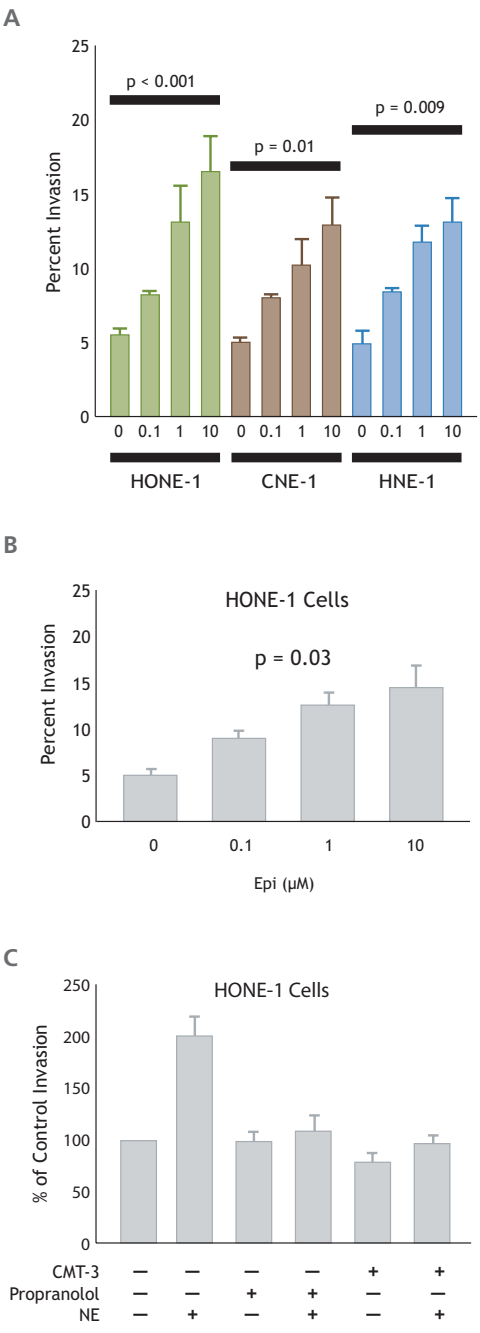
A case of primary superficial-spreading melanoma illustrates intense β2-AR cytoplasmic/membrane immunoreactivity within the balls of melanoma cells occupying the dermal/epidermal junction. The overlying epidermis and underlying dermis are non-immunoreactive (magnification, X400).

FIGURE 5




**(A)** MMP-2, **(B)** MMP-9, and **(C)** VEGF concentrations in medium from HONE-1 clone 39 cells after treatment with norepi. Levels of protein in culture supernatants were measured after treatment with 0.1, 1, and 10 μmol/L norepi for 1, 3, 6, and 12 hours. Values are presented as percent change from untreated HONE-1 cells. Bars, SE.

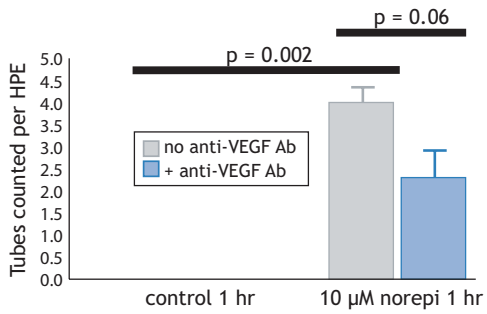
FIGURE 6




**(A)** Membrane invasion culture system (MICS) analysis of NPC cell lines (HONE-1, CNE-1, and HNE-1) after treatment with 0, 0.1, 1.0, and 10 μmol/L norepi (NE). **(B)** MICS analysis of HONE-1 cells after treatment with 0, 0.1, 1.0, and 10 μmol/L epi (Epi). **(C)** Effect of MMP inhibitor (CMT-3) and β-blocker (propranolol) on NPC cell invasion in the presence or absence of norepi. Bars, SE.

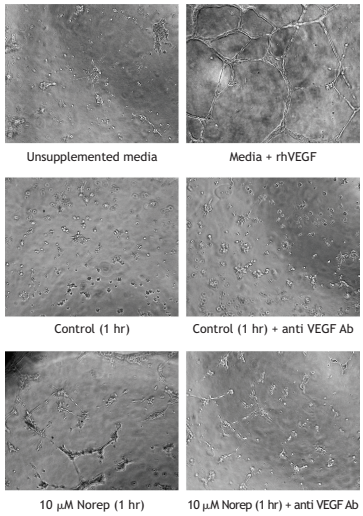
**FIGURE 7** Assessment of the release of biologically active VEGF in culture supernatants by HONE-1 clone 39 cells

A




HONE-1 cells were grown for 1 hr in serum-free media alone (control 1 hr) or with 10 µmol/L norepi (10 µmol/L norepi 1 hr). Angiogenic activity in the culture supernatants was assessed by their ability to induce endothelial cell tube formation in HUVEC. Some HUVECs were coincubated with 2 µg/ml of an anti-VEGF neutralizing antibody (+ anti-VEGF Ab) to assess the role of VEGF in the angiogenic activity of the culture supernatants. Data represents the mean +/- SE of tubes counted from 3 high powered fields from each condition.

B




Representative photomicrographs of HUVEC tube formation after incubation with cell culture supernatants from 10 µmol/L norepi-treated and untreated HONE-1 clone 39 cells.

cells express these receptors (Figures 8A and 8B, p. 46); and that treatment with antagonists to block the binding of norepi to the receptors abrogated the up-regulation of MMP-2 (Figure 8C, p. 46) and MMP-9 (Figure 8D, p. 46); tumor cells in several NPC biopsies examined also express β-ARs (Table 2, p. 47; Figure 9, p. 46). The data suggest that norepi, a stress hormone produced after the activation of the SAM axis, may play a role in the pathogenesis of NPC.[32]

These studies also show that stress may act as a co-factor through the upregulation of molecules produced by tumor cells, such as MMPs, VEGF, and cytokines. These factors act by enhancing the progression of the tumor and increasing the possibility of metastases. Furthermore, this connection of stress with cancer is independent of the role that stress may play in the dysregulation of the cellular and innate immune responses to tumors.

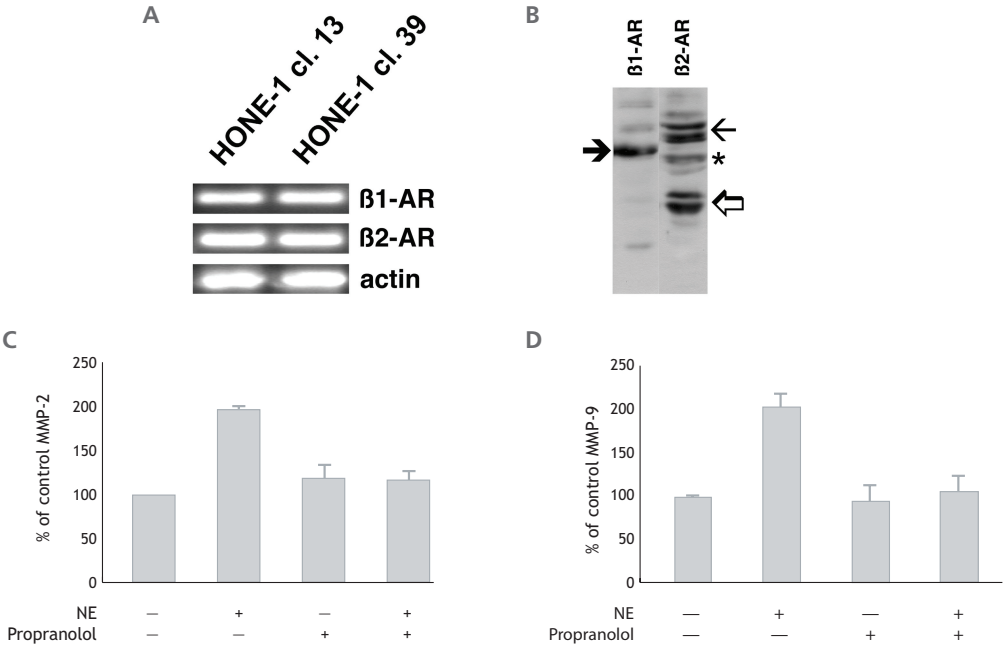
## Future Research Directions

Future directions of this line of research will entail the elucidation of the signaling pathways involved in the stress-related regulation of expression of the pro-angiogenic and prometastatic factors in tumor cells. Furthermore, *in vivo* models studying the effect of various stressors on nude mice bearing NPC and melanoma tumors (using the cell lines previously discussed) will further test hypotheses derived from our *in vitro* studies.[33]

Within the clinical context, we have recently been funded to examine the interplay between depressive symptoms, severe life stressors, and histopathological characteristics and immune reactivity in basal cell carcinoma (BCC).

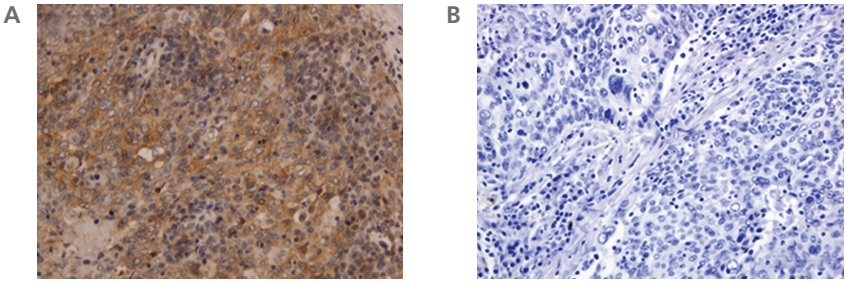
This research builds on more than two decades of work from our laboratory demonstrating links between psychological distress and immune responses. Because BCC is a type of cancer that can induce a tumor-specific immune response, it may provide important information about the impact of stress on the response of the immune system to tumors that express tumor-associated antigens.

**FIGURE 8** Expression of β-ARs in HONE-1 cells




**(A)** RT-PCR analysis of β-ARs (β1-AR and β2-AR) expression in the NPC cell lines, HONE-1 cl. 13 and HONE-1 cl. 39. Actin gene expression in each cell line was utilized to monitor variability in loading. **(B)** Western blot analysis of β1-AR and β2-AR expression in HONE-1 clone 39 cells. Cell lysates probed for β1-AR revealed a band with an apparent molecular weight of 75 x $10^3$ $M_r$ (➔) in lysates of HONE-1 cells. Several bands were observed when cell lysates were probed for β2-AR (β2-AR). Two bands with molecular weights of about 47 to 50 x $10^3$ $M_r$ (⇦) which is consistent with the weight of the unglycosylated protein. The band at about 65 x $10^3$ $M_r$ (✱) is the glycosylated receptor, while the bands at about 90 to 100 x $10^3$ $M_r$ result from dimerization (←). These bands were not observed in blots incubated with normal rabbit serum (not shown). The effect of ß-blocker (propranolol) on MMP-2 **(C)** and MMP-9 **(D)** protein levels in culture supernatants of HONE-1 clone 39 cells in the presence or absence of norepi.

**FIGURE 9** Immunohistochemical analysis of NPC biopsies showing distribution of β2-AR




**(A)** β2-ARs are localized in the plasma membrane of this representative NPC biopsy.
**(B)** Specificity of first antibody was assessed by the absence of reactivity after incubation of an adjacent section with normal mouse IgG used as negative control. Scale bar, 60 μm.

**TABLE 2** The presence of β2-AR on NPC tumor cells in undifferentiated NPC biopsies

| Site of Involvement | Diagnosis — Comments | Morphology | Type | EBV | β2-AR |
|---|---|---|---|---|---|
| Nasopharynx | Lymphoepithelioma | Undiff | 3 | NT | 1+ |
| Naospharynx | Not indicated | Undiff | 3 | + (IHC) | 1+ |
| Nasopharynx | Carcinoma | Undiff | 3 | NT | 2+ |
| Nasopharynx | Non-keratinized | Undiff | 2 | + (IS) | 2+ |
| Nasopharynx | Not indicated | Undiff | 3 | + (IS) | 2+ |
| Nasopharynx | Acute and chronic inflammation | Undiff | 2 | NT | 2+ |
| Nasopharynx | Lymphoid hyperplasia | Undiff | 2-3 | NT | 3+ |
| NT=not tested; IHC=immunohistochemistry; IS=*in situ* hybridization | | | | | |

BCC is the most common type of skin cancer, and although it is generally not fatal, it is a significant public health concern.

This study is the first of its kind to examine the implications of stress/depression for BCC. We will be collecting valuable information about the implications of psychological distress as a possible co-factor for BCC and other tumors.


## Acknowledgements

Dr. Glaser's research has been supported in part by the National Cancer Institute (R01 CA100243 "Stress, the Immune System and Basal Cell Carcinoma") and The Mitchell Endowment and The Ohio State University Comprehensive Cancer Center Core Grant (CA16058).

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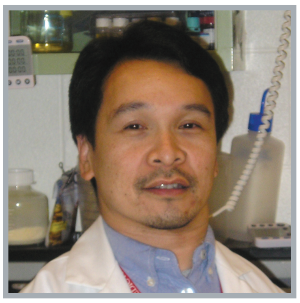
Ronald Glaser, PhD

Dr. Glaser is a Professor of Molecular Virology, Immunology, and Medical Genetics at Ohio State University College of Medicine, and Director of the Ohio State University Institute for Behavioral Medicine Research. He has studied the role of human oncogenic herpesviruses and cancer for several years focusing primarily on Epstein-Barr virus (EBV) latency and the replication of EBV in epithelial cells in order to help understand the role that EBV plays in the etiology of nasopharyngeal carcinoma. He is past Chair of the Department of Medical Microbiology and Immunology, past Associate Dean for Research and Graduate Education in the College of Medicine, and is past Associate Vice President for Research at Ohio State University. Since the early 1980s, he has worked closely with Dr. Janice Kiecolt-Glaser devoting much of his effort to the field of psychoneuroimmunology (PNI) studying the interactions between the central nervous system, the immune system, and the endocrine system; how stress modulates these systems; and the health implications of these interactions.

Dr. Glaser's work has focused on stress and herpesvirus latency, vaccine responses, wound healing, and the role that stress may play as a co-factor in the etiology and progression of malignant disease. More recently, in collaboration with Dr. Yang (a research scientist in his laboratory), he has initiated a line of research focusing on the direct impact of the catecholamine stress hormones, epinephrine, and norepinepherine on tumor progression and angiogenesis. The outcome of these studies thus far has shown that nasopharyngeal carcinoma tumor cells express β-adrenergic receptors and, when treated with the stress hormones, showed an up-regulation of two matrix metalloproteinases, as well as vascular endothelial growth factor. Similar results have been found for melanoma tumor cells.

Dr. Glaser has published 287 articles and book chapters in the area of viral oncology and in the area of stress and immune function. He currently holds the Gilbert and Kathryn Mitchell Endowed Chair in Medicine. Additionally, he is the past President, Psychoneuroimmunology Research Society and President-Elect, Academy for Behavioral Medicine Research. He is also a past Leukemia Society of America Scholar and a AAAS Fellow. He has recently been appointed to the Institute of Medicine Committee on Military Nutritional Research.



Eric Yang, PhD

Dr. Yang is a research scientist in the Institute for Behavioral Medicine Research and an Adjunct Assistant Professor in the Department of Molecular Virology, Immunology, and Medical Genetics at Ohio State University. He is a recent recipient of a Research Supplement to Promote Diversity in Health-Related Research from NCI. His research focuses on the stress-related modulation of tumor progression. His role in the NCI funded project "Stress, the Immune System and Basal Cell Carcinoma" is to examine the relationship between psychological stress/depression, tumor immuno-reactivity, and the expression of matrix metalloproteinases (MMPs) in tumor biopsies from patients with basal cell carcinoma and other tumors. His efforts are also directed at elucidating the role(s) of stress hormones, particularly the catecholamines, norepinephrine and epinephrine in the modulation of the pro-angiogenic and metastatic properties of tumor cell lines.

Dr. Yang received his B.S. degree from Seton Hall University and Ph.D. in Molecular, Cellular, and Developmental Biology from Ohio State University. Prior to joining the laboratory of Dr. Glaser to study the stress-related modulation of MMP expression during cutaneous wound healing, Dr. Yang was a postdoctoral fellow in the laboratory of Dr. Susan V. Bryant at the University of California, Irvine, studying the role of MMPs during salamander limb regeneration.

# Stress and UV-Induced Squamous Cell Carcinoma

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Skin cancer is the most common type of cancer in the United States. Approximately 1 in 5 Americans is likely to develop skin cancer in their lifetime.[1] The majority of skin cancer cases involve non-melanoma skin cancers (NMSC), such as basal or squamous cell carcinoma (SCC). Three million new cases of NMSC occur each year worldwide,[2] with approximately 2,000 deaths per year in the US alone. Ultraviolet radiation (UV) from sunlight is thought to cause more than 90% of skin cancers.[3] Unfortunately, numerous factors have made psychological stress nearly ubiquitous. Chronic stress is known to adversely affect numerous diseases including cancer.[4] In addition to potentially increasing susceptibility to certain types of cancers, chronic stress is significantly increased during cancer diagnosis and treatment and is, therefore, likely to adversely affect treatment outcome.

NMSCs are immunogenic tumors that can be eliminated by anti-tumor immune responses. However, in addition to initiating tumors, UV exposure suppresses protective cell-mediated immunity. Such immunosuppression further increases susceptibility to NMSCs, as well as infectious diseases. Chronic stress suppresses protective immune function. Given the increasing prevalence of NMSCs, it is important to understand whether and how chronic stress and UV exposure may act together to increase susceptibility to disease. Therefore, we investigated potential mediators of a stress-induced increase in the emergence and progression of UV-induced squamous cell carcinoma.

## Research Questions

The studies described here were designed to answer the following questions:[5]

- Does chronic stress increase susceptibility to UV-induced SCC?
- If so, does chronic stress affect the emergence and/or progression and/or regression of SCC?
- What are the mechanisms by which chronic stress affects SCC emergence/progression?

## Experimental Design and Methods

### Animals

SKH1 mice were used (Charles River), ~7 weeks old at the start of the experiment. Housing and all experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC). SKH1 is an ideal strain because UV-induced lesions resemble human SCC.

Animals were maintained on 12-hour light-dark cycle and given food and water ad libitum. The treatment group included:

- no stress + UV,
- chronic stress + UV,
- no stress, no UV.

### Naturalistic model of UV-induced SCC

Mice were exposed to UVB light (1 minimal erythemic dose, 2240 J/m2 (~10 minutes) per session) every Monday, Wednesday, and Friday during weeks 1 to 10. This model of UV exposure does not induce blistering and involves a relatively long (naturalistic) course of time of tumor development.

**Naturalistic model of chronic stress**
Animals were placed in restrainers for 6 hours per day during weeks 4 to 6 of UV exposure. This model of restraint does not involve compression or pain and provides ample ventilation. Body and organ weights of control and chronically stressed mice were equivalent (unlike other chronic stressors that significantly decrease body and organ weights). This well characterized and widely used model simulates a collapsed burrow environment, which induces stress response in burrowing animals.

**Tumor monitoring and quantification**
Animals were monitored weekly (weeks 10 to 35) for papilloma/tumor emergence. Tumor number, size, and location were measured and digitally photographed and body weight was recorded every week.

**Tissue collection**
Animals were euthanized with $CO_2$ at week 35. Whole blood was collected for flow cytometric analysis and quantification of plasma corticosterone. Skin, tumors, and lymph nodes were collected rapidly and frozen for follow-up analyses.

**Immunohistochemistry, quantitative PCR and flow cytometry**
All quantification and analyses were performed on coded sections by "blinded" observation. H&E stained tumor sections were classified by a veterinary pathologist. CD4+, CD8+, and CD25+ leukocytes were detected and quantified by immunohistochemistry. *IL-12*, IFN-γ, *IL-10*, *IL-4*, CD3ε and CTACK gene expression was quantified by real time PCR. CD4+CD25+ suppressor T-cells were quantified in whole blood by flow cytometry.

## Significant Results

**Chronic stress increases susceptibility to SCC**
Chronically stressed mice showed increased tumor size and number compared to controls. Chronically stressed mice showed earlier tumor incidence than nonstressed controls. Nonstressed, control mice showed 30% tumor regression at week 34. Chronically stressed mice showed no regression, but continued tumor progression at week 34 (Table 1).

**Chronic stress decreases Th1 type chemokine and cytokine gene expression**
Chronically stressed mice showed decreased cutaneous T-cell attracting chemokine (CTACK/CCL27) gene expression in lesions that are similar to actinic keratosis in humans. This indicated decreased T-cell chemoattraction in precancerous lesions, a finding that was supported by decreased CD3ε gene expression and immunohistochemistry results indicating reduced CD4+ and CD8+ T-cell infiltration.

**TABLE 1** Chronic Stress ↑ Emergence, ↑ Progression, ↓ Regression of UV-Induced Squamous Cell Carcinoma[5]

| Parameter | No Stress | Chronic Stress | P |
|---|---|---|---|
| Total tumor number | 32 | 84 | .02 |
| Weekly tumor increase | 0.20 | 0.50 | .02 |
| Tumor density | 4.00 (1.84 to 6.16) | 10.5 (6.23 to 14.8) | .09 |
| Tumor area ($mm^2$) | 5.03 (4.22 to 5.84) | 7.29 (6.54 to 8.04) | .02 |
| Median week to first tumor | 16.5 | 15 | .03 |
| Week of 50% incidence | 21 | 15 | N.A. |
| Week of 100% incidence | 34 | 31 | N.A. |
| Tumor regression at week 34 | 31.3% | -16.7% | .02 |

Chronically stressed mice showed decreased *IL-12* and IFN-γ gene expression but no difference in *IL-4* or *IL-10* gene expression, suggesting a shift in cytokine balance toward Type 2/suppressor cytokine mediated immunity that is known to be detrimental for SCC (Table 2).

**Chronic stress decreases T-cell infiltration around tumors**

Chronically stressed mice showed decreased numbers of CD4+ T-cells compared to controls. Similar data were observed for CD8+ T-cells (Figure 1).[5]

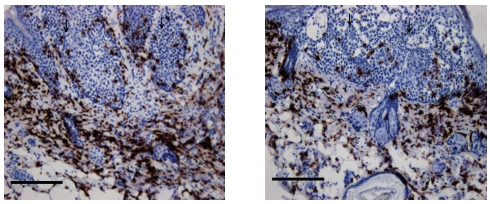
**Chronic stress increases suppressor T-cell infiltration**

Chronically stressed mice showed increased numbers of CD25+ suppressor cells within and around tumors compared to non-stressed controls (Figures 2 and 3, p. 56). Chronically stressed mice also showed increased numbers of CD25+ suppressor T-cells in the systemic circulation (Figure 4, p. 57).

**Model for chronic stress induced susceptibility to SCC**

Based on our findings, we propose a model to describe how chronic stress significantly

**FIGURE 1** Chronic stress ↓↓ CD4 T-cell infiltration[5]



NS CD4+ Chr STR CD4+

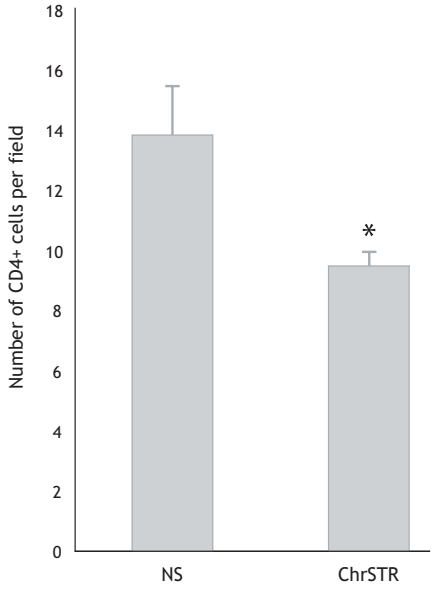



**TABLE 2** Chronic Stress ↓ CTACK (CCL27), CD3e, *IL-12* & IFN-γ Gene Expression in Precancerous Lesions[5]

| | No Stress | Chronic Stress | |
|---|---|---|---|
| CTACK | 142.28 | 101.05* | Th1 cytokines suppressed |
| (sem) | 10.00 | 11.00 | |
| CD3e | 0.36 | 0.18* | |
| | 0.04 | 0.04 | |
| *IL-12* | 0.11 | 0.05 | |
| | 0.02 | 0.01 | |
| IFNg | 0.07 | 0.03* | |
| | 0.01 | 0.01 | |
| *IL-4* | 0.07 | 0.09 | Th2 cytokines no effect |
| | 0.02 | 0.07 | |
| *IL-10* | 0.18 | 0.13 | |
| | 0.03 | 0.04 | |

**FIGURE 2** Chronic stress ↑↑ CD25+ suppressor cell infiltration ⇒ Chronic stress ↑↑ active immuno-suppression[5]

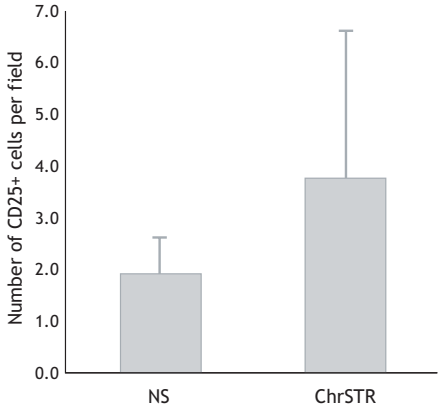
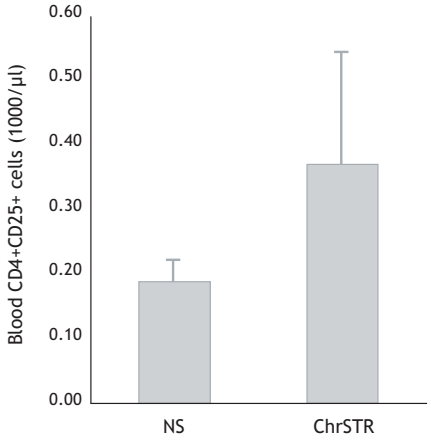



**FIGURE 3** Chronic stress ↑↑ CD25+CD4+ suppressor T cell numbers in systematic circulation[5]




increases susceptibility to SCC (Figure 4, p. 57):

1. Chronic stress suppresses CTACK gene expression.
2. This decreases chemoattractive signals that direct T-cells to the skin and therefore reduces T-cell infiltration at the time of UV exposure.
3. Decreased T-cell infiltration is accompanied by suppressed Type 1 cytokine (*IL-12* & IFN-γ) production in chronically stressed animals compared to controls.
4. In addition to suppressing protective Th1 type immunity, chronic stress increases CD25+ suppressor T-cells and thus mobilizes a potent mechanism for active immunosuppression. By suppressing T-cell infiltration at the time of UV exposure, chronic stress induces long-lasting effects that are registered months later even in the absence of continued stress. The collective effects of chronic stress significantly suppress protective immunity resulting in increased emergence and progression of SCC.

*The collective effects of chronic stress significantly suppress protective immunity resulting in increased emergence and progression of SCC.*

## Overall Conclusion

- Chronic stress increases susceptibility to skin cancer and shifts the balance from protective to suppressive immune responses.
- On the one hand, chronic stress suppresses Type 1 cytokines and CCL27/CTACK gene expression, and CD4+ and CD8+ T-cell infiltration at sites of tumor emergence and progression, while on the other it increases the numbers of regulatory/suppressor cells at tumor sites and in circulation.

- To our knowledge, these results are the first to show that chronic stressors mobilize endogenous immunosuppressive mechanisms like regulatory/suppressor T-cells.
- **The detrimental effects of stress on critical clinical, cellular, and molecular parameters, are observed months after the cessation of stress.**

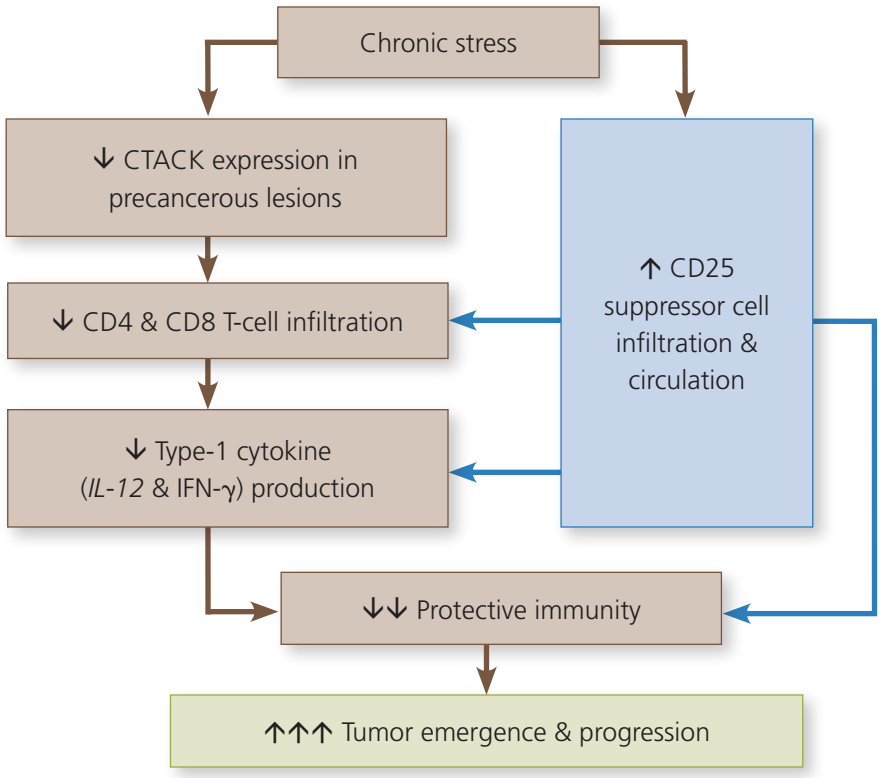
### Future Research Directions

- Elucidate endocrine and immune mechanisms by which chronic stress suppresses CTACK gene expression and T-cell chemoattraction and homing to the skin.
- Elucidate mechanisms by which chronic stress increases suppressor T-cell numbers and activity.

*To our knowledge, these results are the first to show that chronic stressors mobilize endogenous immunosuppressive mechanisms like regulatory/ suppressor T-cells.*

- Examine the effects of chronic stress on DNA repair following UV-induced DNA damage.

**FIGURE 4** Effects of chronic stress manifest 9 minutes after cessation of stress



## Implications for Cancer Control

- Stress pervades almost all aspects of life and is especially salient during diagnosis, treatment, and follow-up for cancer and other diseases.
- Therefore, our findings may be relevant for conditions where chronic stress may increase initial susceptibility to cancer, decrease effectiveness of tumorimmunotherapy, or contribute to systemic immunosuppression during cancer treatment.
- Knowledge gained from transdisciplinary studies such as this, which examines cancer in a biobehavioral context, will increase the accuracy and timeliness of risk evaluation, improve preventative and therapeutic interventions, and help optimize a patient's response to treatment.

## Acknowledgements

This research has been supported by the National Cancer Institute (RO1 CA107498 "Stress & UV-induced Squamous Cell Carcinoma").

This project has been the major focus of the doctoral dissertation of Dr. Dhabar's graduate student, Ms. Alison Saul. Ms. Saul is slated to defend her dissertation on February 27, 2007, and to receive her Ph.D. in March 2007. We are grateful for this meaningful and significant accomplishment that was made possible by funding that supported this project and Ms. Saul.

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## Invited Presentations

Dhabhar, F.S. (2005) Effects of stress on squamous cell carcinoma. *American Association for Cancer Research – Frontiers In Cancer Prevention*, (Baltimore, MD, October 30 – November 2, 2005).

Dhabhar, F.S. (2006) Enhancing versus suppressive effects of stress on skin immune function. *American Academy of Dermatology 64th Annual Meeting: Forum on Stress, The Nervous System, & Cutaneous Immunology*, (San Francisco, CA, March 3-7, 2006).

Dhabhar, F.S. (2006) Effects of stress on skin immunity and skin cancer. *Cornell University Stress & Skin Symposium*, (New York, NY, June 2-4, 2006).

## Press & Media Reports

WebMD, Dec. 6, 2005, *Stress Raises Skin Cancer Risk*, Miranda Hitti, USA.

KABC, Los Angeles, Dec. 7, 2005, *Stress and Skin Cancer*, Denise Dador, USA.

NBC News, KDSK, MO, Dec. 7, 2005, *Stress Combined With Exposure Makes Skin Cancer*, USA.

Dermatology Times, Feb 1, 2006, *Stress & Illness Link Alive & Well*, Beth Kapes, USA.

Ladies' Home Journal, Oct. 2006, *The Last Stress-Survival Guide You'll Ever Need*, Diane Cole, USA.

## Firdaus S. Dhabhar, PhD

Dr. Dhabhar is Associate Professor of Psychiatry & Behavioral Sciences at Stanford University, and is the Director of Research at the Stanford Center on Stress and Health. He is a member of the Stanford Cancer Center and holds a joint appointment in the Immunology Program. He also holds an adjunct appointment in the Laboratory of Neuroendocrinology at The Rockefeller University, from where he received his Ph.D. in Biomedical Sciences. Dr. Dhabhar's laboratory has elucidated psycho-physiological, cellular, and molecular mechanisms by which acute versus chronic stressors respectively enhance or suppress in vivo immune responses. His laboratory's findings have spurred interest in the newly appreciated immunoenhancing and potentially health-promoting aspects of acute or short term stress.

Dr. Dhabhar's laboratory is engaged in both pre-clinical and clinical studies designed to examine the positive or negative effects of acute versus chronic stressors and psychological disorders on immune function and health. A major part of his research effort is focused on identifying pathways through which hormones, immune cells, cytokines, and chemokines interact to mediate the effects of psychological stress on susceptibility/resistance to UV-induced squamous cell carcinoma.

Dr. Dhabhar has received numerous honors and awards including the Council of Graduate Schools Distinguished Dissertation Award and the PsychoNeuroImmunology Research Society Young Investigator Award. Dr. Dhabhar has served on three committees at The National Academies, is a grant reviewer for the NIH, and serves as an elected member on the Scientific Council of the Psychoneuroimmunology Research Society.

# Biobehavioral Cytokine Interactions in Ovarian Cancer

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Ovarian cancer is the second most common gynecologic cancer. Although women diagnosed with localized disease have a 95% likelihood of 5-year survival, the majority of women with epithelial ovarian cancer are diagnosed with advanced stage disease. Their 5-year survival rates can be less than 25% for those diagnosed with distant disease. Because of low rates of survival for the majority of women with ovarian cancer, identification of factors contributing to tumor growth and disease progression is of paramount significance.

*This was the first report in the literature of which we were aware of a relationship found between a psychosocial factor and the immune response in the tumor microenvironment.*

Substantial evidence suggests that psychosocial factors such as stress, depression, and social support are able to modulate many of the immunologic activities relevant to cancer.[1–3] However, little is known about other mechanisms by which biobehavioral mechanisms may influence growth and progression of cancer.

Much of the research investigating relationships between behavioral factors and oncology has focused on the immunosuppressive effects of distress on both the innate and adaptive immune responses. However, there are many other pathways where links may exist between biobehavioral factors and tumor progression. For example, angiogenesis, the formation of new blood vessels that enhance tumor growth, is a key process in the growth of most solid tumors and their metastatic spread. There has been little investigation of associations between psychosocial factors and cytokines involved in the promotion or inhibition of angiogenesis. Stimulation of such pathways by stress hormones could potentially contribute to tumor progression.

## What We Know/Evolution of this Research Program

Currently recognized risk factors explain only a modest percentage of all ovarian cancers. A substantial body of research has emerged over the last 30 years, documenting relationships between behavioral factors, cancer risk, and disease progression.[4–14] However, the inconsistent nature of the findings has left controversy regarding the strength of these relationships.[3, 15–19] There are indications from the literature that patients with sustained stress or emotional distress have poorer survival rates.

A recent large-scale epidemiologic study reported that those cancer patients having emotional difficulties prior to the diagnosis of cancer were more likely to have a) sustained depressive symptoms

following diagnosis and b) a 2.6 times greater hazard of dying over a 15-month period than patients without previous difficulties. Patients experiencing depression for the first time after diagnosis had no poorer survival rates than those without depression, suggesting that prior emotional difficulties may be a risk factor for negative outcomes during cancer diagnosis and treatment.[7]

Several prospective studies have reported relationships between long-term distress or depression and cancer progression, although findings are not consistent.[20–22] Higher levels of distress and/or lower levels of social support have also been associated with blunted functioning of both adaptive and innate immune cells in peripheral blood. For example, early stage breast cancer patients reporting greater stress between surgery and adjuvant therapy had lower levels of natural killer (NK) cell activity, diminished response of NK cells to recombinant IFNγ, and decreased proliferative response to mitogens, controlling for variables that could potentially affect the immune response, such as age, disease stage, and days since surgery.[23]

Another study among women with early stage breast cancer who are 1 to 2 months post-surgery found that positive factors such as perceived social support, use of positive reframing, and optimism were associated with a greater T-cell proliferative response. Moreover, early stage breast cancer patients participating in stress management interventions showed significantly increased T-cell functioning compared to wait-list controls in two separate studies.[24–26]

Up to the time of our initial studies, investigation of relationships among psychosocial factors and immunity in gynecologic cancers had been minimal.[27] Little was known about whether there were links between biobehavioral factors and the immune response in the tumor microenvironment. This question was a critical one because of the general down-regulation of the immune response in the tumor microenvironment, and the fact that this was the relevant environment for analysis of tumor-immune interactions.

This research began with an R21 grant to study relationships between social support, depressed mood, and the innate and adaptive immune response in peripheral blood and in the tumor microenvironment in ovarian cancer patients. The control group for this study was a group of patients with suspected ovarian cancer who turned out at the time of surgery to have benign histology. Levels of distress in ovarian cancer patients and benign patients were both elevated and approximately equivalent presurgery, and that as expected, NK cell cytotoxicity of ovarian cancer patients was substantially less than that of benign patients.[28] Also the NK cytotoxicity in ovarian cancer patients was found to be significantly impaired in the tumor microenvironment (Figure 1).

Interestingly, higher levels of social support were related to higher levels of NK cytotoxicity in lymphocytes isolated from the tumor, whereas higher levels of distress were related to lower levels of NK cytotoxicity in the tumor microenvironment. This was the first report in the literature of which we were aware of a relationship found between a psychosocial factor and

**FIGURE 1** NK Cytotoxicity in 3 compartments in ovarian cancer

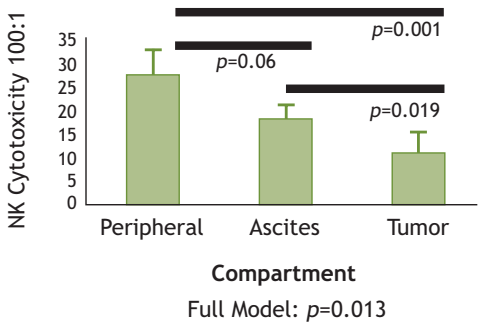
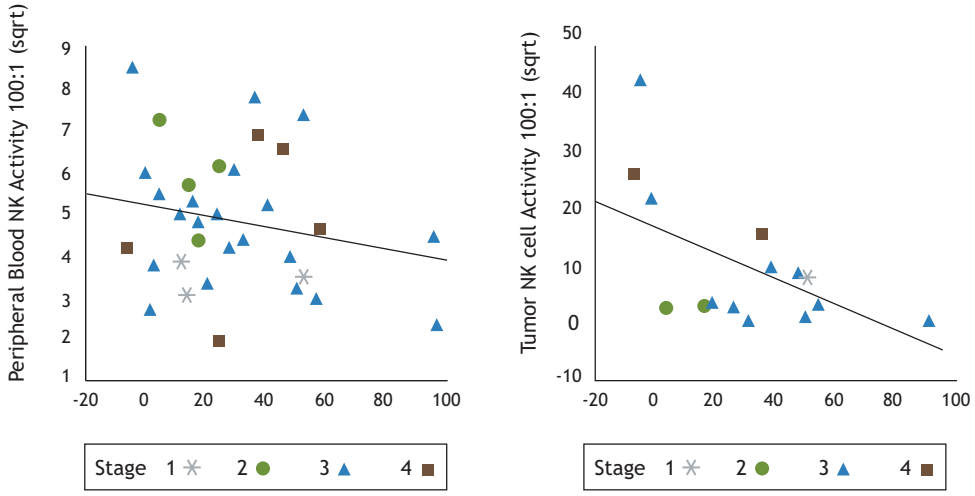


**FIGURE 2** POMS distress and NKCC (100:1) in PBL and tumor



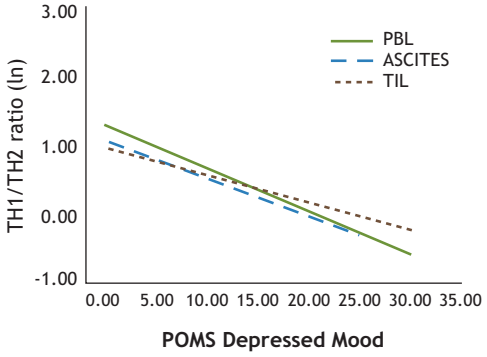

*Covariate: stage*

*Lutgendorf et al., JCO, 2005*

cellular immune response in the tumor microenvironment (Figure 2).

With respect to adaptive immunity, our recent findings (under review) indicate that greater depressed mood is also related to a greater shift from autologous-tumor stimulated T-cells expressing Type-1 cytokines (IFNγ) toward Type-2 cytokine expressing T-cell populations *(IL-4)* averaged across all compartments (Figure 3). Thus a down regulation is seen of both adaptive and innate immunity in ovarian cancer patients with depressed mood.

**FIGURE 3** Depressed mood and T-cell function in 3 compartments in ovarian cancer patients




## Development of a Broader Biobehavioral Model

In the course of this work we started to ask whether other factors specifically related to tumor growth might be linked with psychosocial factors. Because of previous work done in our lab linking stress and an inflammatory cytokine related to mortality and morbidity called interleukin-6 in older women, we looked at interleukin-6 and several psychosocial factors in our ovarian cancer patients.

We found that advanced ovarian cancer patients with higher levels of social support had lower levels of interleukin-6 in two separate studies (Figure 4, p. 64). Furthermore, these relationships were seen not only in peripheral blood but also in ascites.[29] Interestingly, *IL-6* in ovarian cancer patients is also related to prognosis, angiogenesis, and invasiveness of cancers,

suggesting that there are links between behavioral factors and an important cytokine related to cancer progression.

It was around this time that I started collaborating with Dr. Sood, who was studying tumor angiogenesis. Based on this work, we started examining an angiogenic cytokine that is induced by *IL-6*, namely vascular endothelial growth factor (or VEGF).

We examined relationships between social support and VEGF in a small group of ovarian cancer patients and found that those patients who had higher levels of social support had lower serum VEGF (Figure 5, p. 65), and conversely, those with a greater sense of helplessness or worthlessness had higher levels of VEGF.[30] Although these findings were in a small sample of 24 patients, they were sufficiently intriguing to cause us to want to examine mechanisms that might underlie these relationships. At this point, Dr. Steven Cole helped us develop an *in vitro* model whereby we could test the effects of several stress hormones on ovarian cancer cells to examine the production of VEGF and other angiogenic cytokines.

**This work revealed that stress hormones such as catecholamines (norepinephrine, epinephrine, and isoproterenol — a nonspecific beta agonist) all elicited large increments in VEGF from several types of ovarian cancer cell lines, that these effects were blocked by the β-blocker propranolol,** (Figure 6, p. 65)**. We also found that β-adrenergic receptors existed on these cell lines, which could account for the transmission of signals required to elicit these effects.[31]**

These findings formed the basis of my current line of research, which is examining:

1. Psychosocial factors and angiogenic cytokines in peripheral blood in a larger sample of ovarian cancer patients using a panel of angiogenic markers;
2. Whether there is a relationship between psychosocial factors and angiogenic cytokines in the ascites (fluid around the tumor) and in tumor;
3. Whether psychosocial factors are related to clinical disease course in ovarian cancer, and whether angiogenic cytokines mediate this relationship; and
4. Whether the neuroendocrine stress hormones mediate relationships between psychosocial factors and angiogenic cytokines.

**FIGURE 4** Social support and *IL-6* in 2 compartments in advanced ovarian cancer patients

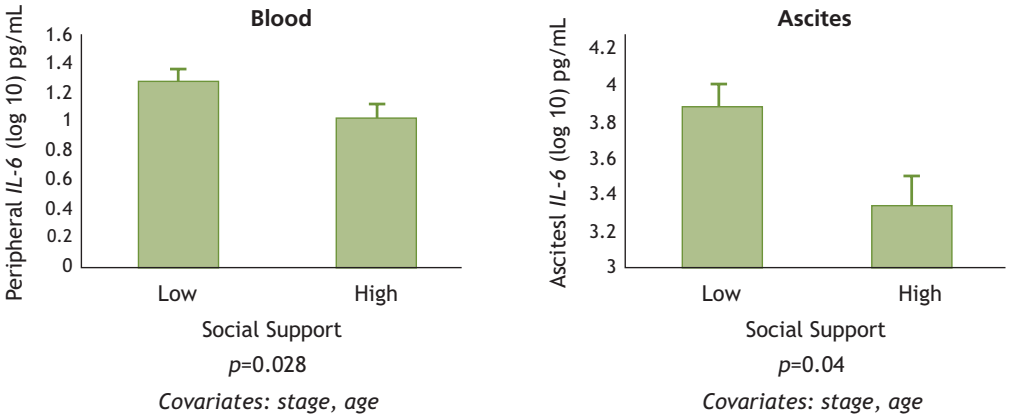
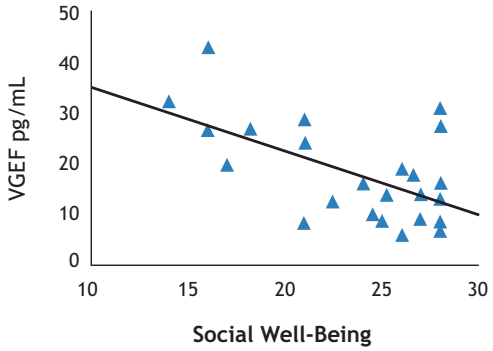
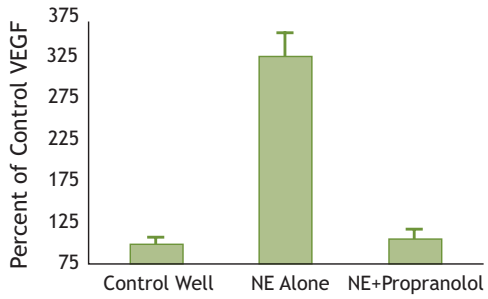


**FIGURE 5** Social well-being and VEGF




*Analyses control for cancer stage*
β = -.57, p = .005
F (2, 21) = 5.71, p = .01

**FIGURE 6** NE-induced VEGF production from SKOV3 cells and blocking




*Covariates: stage, age*

Because of the strong links between adrenergic stress hormones and angiogenic cytokines, we also examined whether catecholamines in tumor tissue were related to psychosocial factors to enable examination of the catecholamines that were biologically available to these tumor cells and would likely contribute to their production of pro-angiogenic cytokines. This work was supported by a Roadmap methodological supplement.

## Methods Used

We measured cytokines in peripheral blood and in ascites, the fluid around the tumor, by ELISA and have measured cytokines in tumor by immunohistochemistry. Salivary cortisol has been measured by radioimmunoassay and catecholamines measured by HPLC electrochemical detection. Psychosocial factors were measured by self-report.

## Results

Our current findings, in a sample of 126 ovarian cancer patients indicate that higher levels of depression are associated with higher levels of *IL-6* in both plasma (r=.29, p=.001) and in ascites (r=.36, p=.003) (Figure 7, p. 66) and with VEGF in serum (r=.23, p=.03) after adjusting for tumor stage (Figure 8, p. 66). There is a marginal positive relationship with *IL-8*. Furthermore, these cytokines are related more strongly with the vegetative symptoms of depression than to depressed affect. These findings are extremely intriguing.

It is known that high plasma levels of *IL-6* can induce "sickness behaviors," fatigue, anhedonia, difficulty with concentration. It is also known that depressed patients have high levels of *IL-6*. These findings suggest the intriguing hypothesis that the tumor production of the angiogenic cytokine *IL-6* may actually be contributing to depressive symptoms in these patients. The causality of these relationships is not known, as they are only correlational. It is possible that depressive symptoms may be contributing to the elevated levels of the angiogenic cytokines in these patients. It is also possible that other factors may be contributing to both depression and to *IL-6*. For example, our depressed ovarian cancer patients also have significantly higher levels of night cortisol, and lower cortisol variability between morning and evening, suggesting a dysregulated diurnal cortisol pattern.

## Tumor Norepinephrine

In addition, ovarian cancer patients with higher levels of depression, as measured by the Center for Epidemiological Studies Depression Scale, have higher levels of tumor norepinephrine for

**FIGURE 7** CESD depression and *IL-6* in 2 compartments in ovarian cancer patients

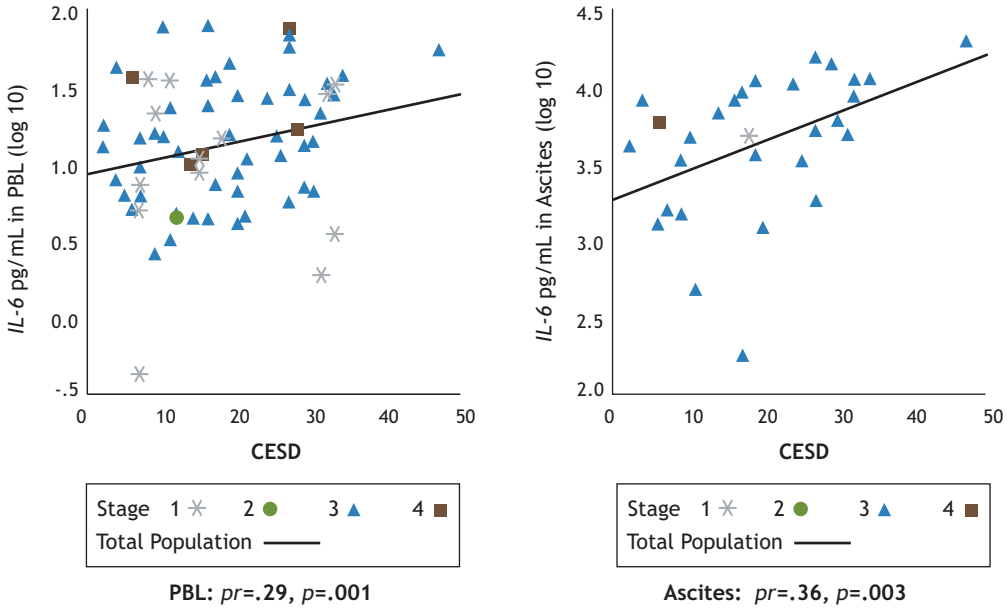
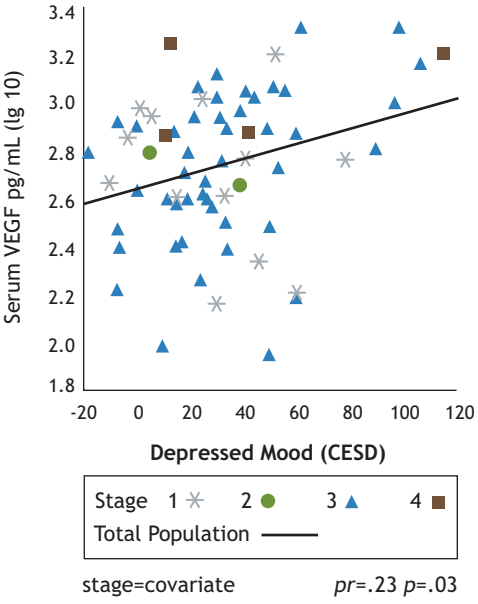



**FIGURE 8** Depressed mood and serum VEGF




both overall depression (r=.32, p=.013) and vegetative symptoms of depression (r=.30, p=.028). In contrast, total perceived social support is negatively correlated with tumor norepinephrine (r=-.28, p=.03). There are no relationships between plasma norepinephrine and tumor norepinephrine, or between depression and plasma norepinephrine, suggesting the importance of measurements of biologically available tumor norepinephrine levels. Our ongoing work consists of comparing these tumor NE levels to levels of cytokines expressed in the tumors as assessed by immunohistochemistry.

## Next Steps/Future Directions

These results show strong relationships between psychological states and neurohormones such as norepinephrine at the tumor level. As these neurohormones are able to influence various processes involved in tumor growth and progression, these findings are consistent with the hypothesis that biological processes

associated with depressive symptoms can contribute to tumor growth. Similarly, angiogenic cytokines produced by tumor cells may support depression in cancer patients.

These findings are really just the beginning of a new area of study where we plan to use bioinformatics approaches to understand relationships between stress and depressive mood and processes underlying tumor growth. These findings also point to the importance of testing β-blockers and *IL-6* antagonists in the treatment of cancer along with inhibitors of other angiogenic molecules. Stress management and complementary approaches that decrease catecholamines and other stress hormones and normalize cortisol would also be important to utilize in this setting. These findings also point to the potential importance of understanding the biological bases of depression of patients with extensive tumor angiogenesis.

*These findings suggest the intriguing hypothesis that the tumor production of the angiogenic cytokine* IL-6 *may actually be contributing to depressive symptoms in these patients. The causality of these relationships is not known, as they are only correlational. It is possible that depressive symptoms may be contributing to the elevated levels of the angiogenic cytokines in these patients.*


## Acknowledgements

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## Susan K. Lutgendorf, PhD

Dr. Susan Lutgendorf is a Professor in the Departments of Psychology, Obstetrics and Gynecology, and Urology and a member of the Holden Comprehensive Cancer Center at the University of Iowa. She received her Ph.D. in Clinical Health Psychology from the University of Miami under a predoctoral training grant fellowship in Behavioral Immunology from NIH. Her postdoctoral training was completed at the University of Iowa Center on Aging, supported by a National Research Service Award postdoctoral fellowship from the NIH.

Dr. Lutgendorf's work focuses on relationships between biobehavioral factors and tumor growth in ovarian cancer. Her current work, funded by NCI, examines how factors such as stress, depression, and social support are linked to biological processes involved in angiogenesis and recurrence in ovarian cancer patients. Her work also investigates relationships between these biobehavioral factors and inflammatory processes in ovarian cancer. Dr. Lutgendorf also has a strong interest in effects of stress management and complementary interventions on quality of life and theimmune response in cancer patients. This work is funded by the National Center for Complementary and Alternative Medicine and by NCI.

Dr. Lutgendorf serves on the editorial boards of *Brain, Behavior, and Immunity*, *Annals of Behavioral Medicine*, and the *International Journal of Behavioral Medicine*. She also has been active in the Psychoneuroimmunology Research Society and the American Psychosomatic Society. She is a member of the Biobehavioral Mechanisms of Emotions, Stress, and Health (MESH) Study Section at NIH. Dr. Lutgendorf's work has been recognized by a New Investigator Award from the Psychoneuroimmunology Research Society in 2004, an Early Career Award from the American Psychosomatic Society in 2002, and by an award from the American Psychological Association, Division 38 (Health Psychology) for Outstanding Contributions to Health Psychology in the year 2000. She received an Outstanding Mentoring Award from the Graduate College of the University of Iowa in 2002.

# Preventing Cancer Metastases Following Surgical Removal of the Primary Tumor: Physiological, Psychological, and Immunological Interventions

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The greatest threat to cancer patients is commonly the recurrence of the disease, rather than the damage associated with the often removable primary tumor. Usually, upon the detection of a solid tumor, a patient undergoes surgical removal of the primary cancer, which is an imperative procedure for extracting the major bulk of mutating and metastasizing malignant cells.

Unfortunately, in approximately half of the patients cancer reappears in the form of metastases, which originate from preexisting micrometastases and from cancer cells released from the primary tumor or its vasculature before or during surgery. These metastases are harder to remove surgically, are more resistant to chemotherapy, and are the major cause of death in cancer patients.

Paradoxically, although the removal of the primary tumor is indispensable, the excision of the tumor is believed to promote the occurrence and growth of metastases via several mechanisms that act synergistically during the immediate perioperative period.[1] Thus, identifying these mechanisms and blocking their deleterious impact during the critical perioperative period may significantly reduce long-term recurrence and improve survival rates in cancer patients.

## Perioperative Processes that Promote Tumor Progression

The above unfortunate consequences of excising the primary tumor are ascribed to several physiological sequels of the procedure, which we have recently reviewed.[1]

For example:

- The mechanical manipulation of the tumor or its vasculature during surgery releases tumor cells into the blood stream and the lymphatics;
- Several processes facilitate the invasiveness, the vascularization, and the growth of tumor cells and of preexisting micrometastases.

*We provided strong evidence that suppression of NK activity, specifically MP-NK activity, mediates the tumor-promoting effects of stress, catecholamines, prostaglandins, and surgery in some tumor models.*

Specifically, the removal of the primary tumor induces a reduction in the levels of anti-angiogenic factors; the tissue damage inflicted by surgery induces the secretion of growth factors; and the release of catecholamines and prostaglandins following surgery increases the invasiveness of tumor cells[2] and promotes the release of pro-angiogenic factors by malignant tissue (e.g. VEGF).[3,4] Collectively these processes cause a pro-angiogenic shift that facilitates the vascularization of metastases, enables blood supply, and delivers a "growth signal" to residual malignant tissue:

- The physiological trauma of surgery and the accompanied nociception, pain, and psychological distress suppress various aspects of the immune system, specifically those known to act against metastases (e.g. NK, CTL).[5,6]
- Opiates administered during and following the operation have been shown to suppress cellular immune functions[7] and to directly promote the growth of human cancer cells and release of pro-angiogenic factors by these tumors.[8]
- All the above processes occur simultaneously during and immediately after surgery, and seem to act synergistically in rendering the patient more susceptible to initiation of new metastases and to a flare-up of preexisting dormant micrometastases (Figure 1).[1,5]

## Research Questions

Our studies during the last decade focused on elucidating mechanisms underlying postoperative immune suppression and tumor promotion, and on devising prophylactic measures to circumvent these undesirable outcomes. Specifically, we attempted to identify:

- Aspects of the surgical procedure that contribute to immune suppression, such as specific anesthetic agents, the degree of tissue damage, and psychological distress.
- Mediating neuroendocrine and paracrine mechanisms of immune suppression and tumor promotion. These could include various hormones, cytokines, and specific aspects of cellular immunity.

**FIGURE 1** The perioperative period: An increased risk of metastasis and an opportunity to eliminate minimal residual disease (MRD)

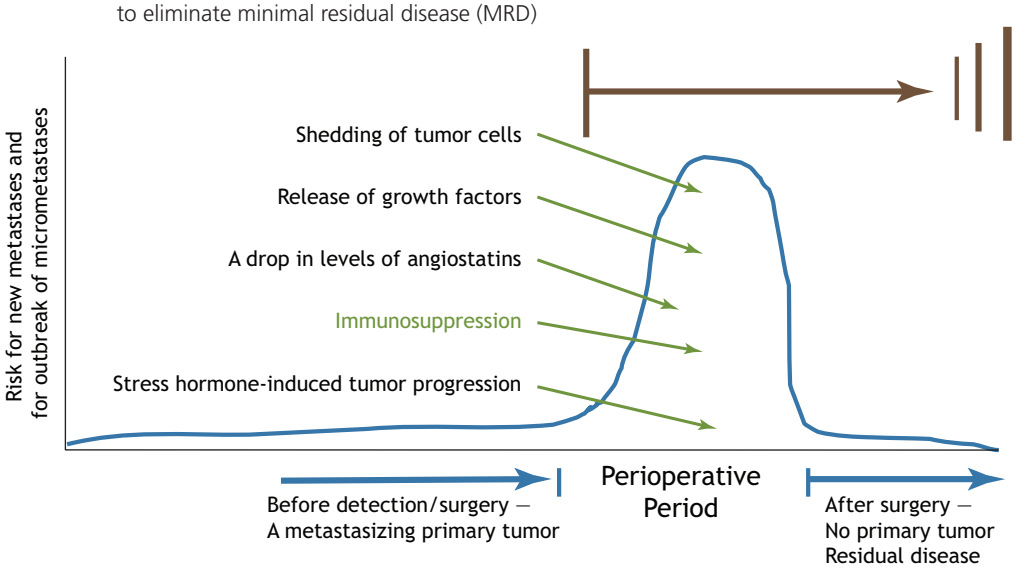
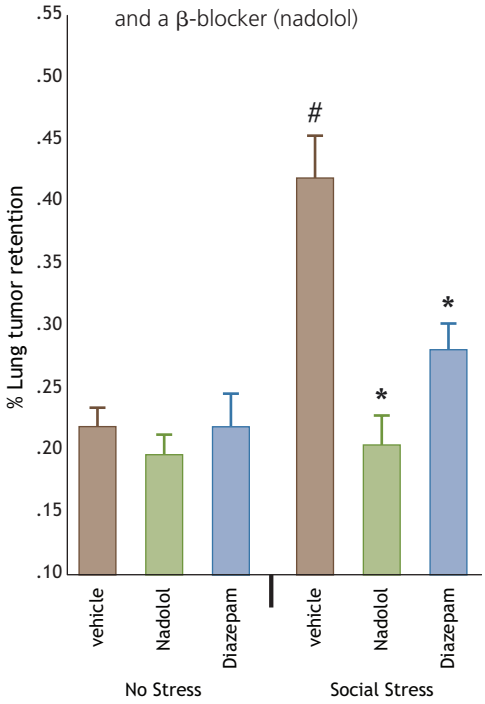


**FIGURE 2** The effect of social stress and its blockade by an anxiolytic (diazepam) and a β-blocker (nadolol)




- Prophylactic approaches to overcome immune suppression and tumor promotion by stress and surgery. These include immunological, pharmacological, and psychopharmacological interventions.

## Methods Used

We conducted studies both in animals and in patients undergoing surgery or subjected to psychological stress. Behavioral, neuroendocrine, and immunological indices include:

- Measures of sickness behavior and physical activity;
- Levels of stress and sex hormones;
- Serum levels of cytokines (e.g., Th1, Th2, pro-/anti-inflammatory) and of their *ex vivo* induced production;
- FACS-based characterization of leukocyte subtypes and their surface and intracellular marker determinants, including receptors, activation markers, and ligand content (e.g., granzyme-b or IFNγ); and
- NK cell numbers and cytotoxicity in the circulation and in the marginating pulmonary compartment.

Cancer progression indices (in rats and mice only) include:

- Resistance to syngeneic experimental metastasis of various tumor types;
- Resistance to syngeneic leukemia progression; and
- Resistance to spontaneous metastasis following excision of several types of syngeneic primary tumors.

## Significant Results from Our Studies

We found the following aspects of surgery to suppress natural killer cell activity (per NK cell) and to promote tumor progression:

- Stress and/or anxiety—we were able to block the deleterious effects of several stress/anxiety paradigms in rats, including social stress (Figure 2), by using β-adrenergic blockers and diazepam.[12]
- Anesthetic and analgesic agents, including fentanyl, ketamine, thiopental, and halothane, but not propofol.[7,9]

**FIGURE 3** Spinal block reduces the promotion of metastasis by surgery

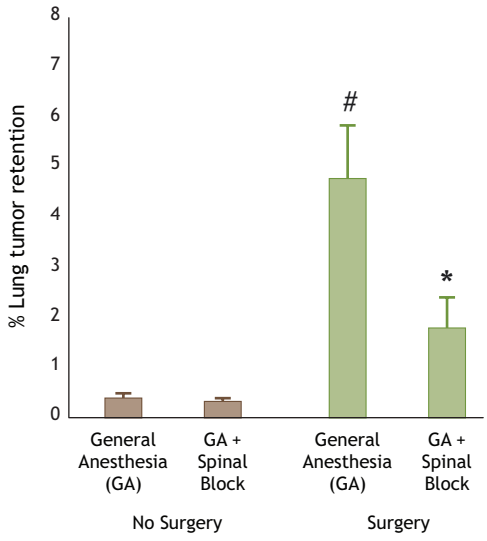
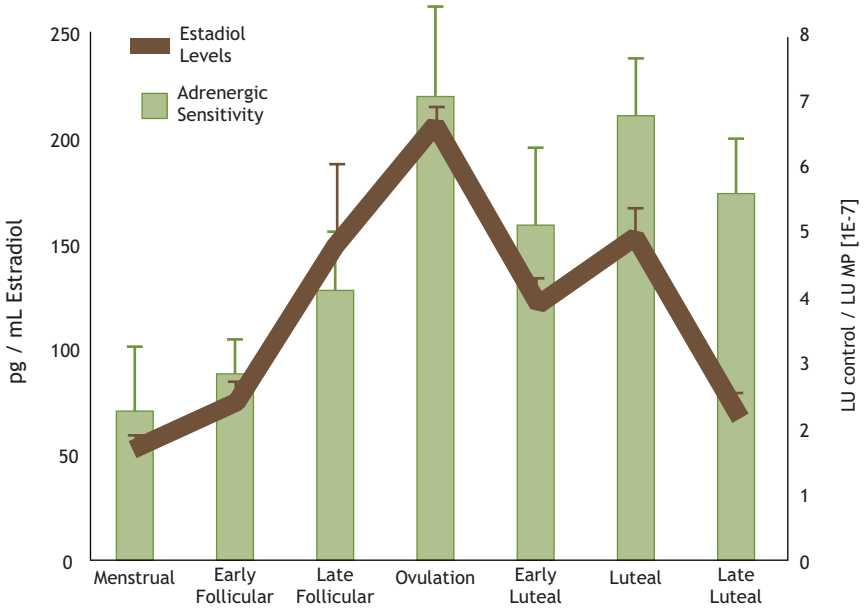
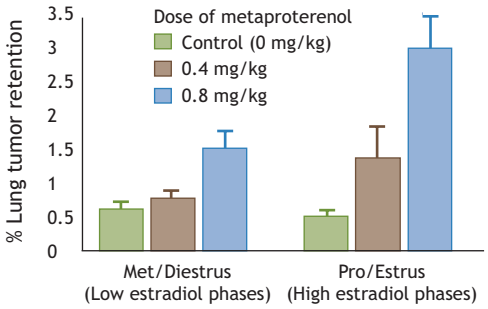


**FIGURE 4** The menstrual cycle modulates the sensitivity of women's NK activity to suppression by a β-adrenergic agonist




- Severe hypothermia (core temperature of 30ºC for 3 hr), but not mild hypothermia (1 hr in 35ºC).[10]
- Tissue damage and nociception—the larger the incision, the greater the effects observed. Several pain alleviating approaches reduced the effects of surgery; most effective was spinal block (Figure 3, p. 73).[11]

**FIGURE 5** The estrous cycle in rats modulate the metastasis-promoting effects of a β-adrenergic agonist (metaproterenol)

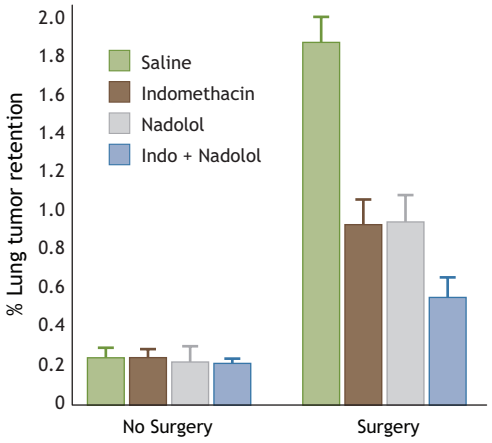



- Blood transfusion—surprisingly our ongoing studies indicate that stored red blood cells, rather than the suspected WBC, jeopardized naïve and operated rats' resistance to MADB106 metastasis and to CRNK-16 leukemia.
- The menstrual cycle was shown, in both female rats and in women (Figure 4), to modulate the suppression on NK activity by catecholamines, and to regulate susceptibility to metastasis (in rats) following surgery and pharmacological stress (Figure 5).[13,14]

Neuroendocrine, paracrine, and immunological mediators—the following were identified:

- We implicated catecholamines and prostaglandins in mediating many of the above effects of stress and surgery (Figure 6, p. 75).[15–17] These factors were found to be sufficient and necessary mediators of the effects of surgery, and their blockade during the perioperative period almost completely abrogated immune suppression and tumor promotion (rodent studies).

**FIGURE 6** The prostaglandin synthesis inhibitor, indomethacin, and the β-blocker, nadolol, reduce the promotion of metastasis by surgery




- We identified a uniquely potent endogenous population of NK cells, marginating-pulmonary NK cells (MP-NK cells). These cells reside in the lung capillaries and are well located to scan circulating cells and to interact with circulating malignant cells. Among other characteristics, MP-NK cells exhibit higher proportion of large NK cells (Figure 7), higher cytotoxicity against standard target cells, and constitute the only population known to exhibit NK cytotoxicity against the syngeneic MADB106 tumor line.[17,18] If such a population exists in humans, then many tumors that are considered "NK-resistant" are actually under control of MP-NK cells, and the role played by cellular immunity in controlling metastasis is greater than currently assumed.
- We provided strong evidence that suppression of NK activity, specifically MP-NK activity, mediates the tumor-promoting effects of stress, catecholamines, prostaglandins, and surgery in some tumor models (rodent studies).[15-17,19,20]
- In patients undergoing various types of surgery we found marked suppression of several aspects of immunity even before surgery, and additional and independent suppression following

**FIGURE 7** FACS analyses of small vs. big NK cells in the blood and marginating pulmonary compartments

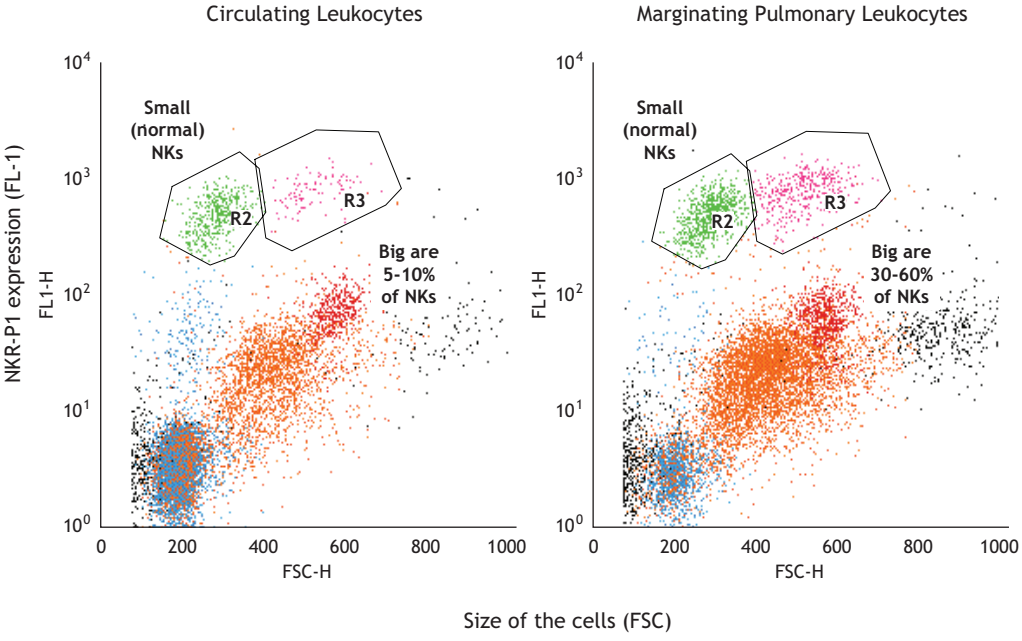
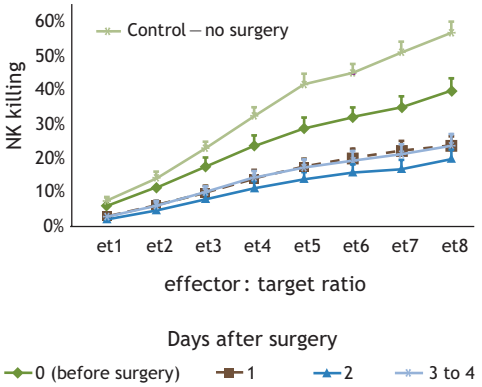


**FIGURE 8** NK activity in matched controls and in patients before surgery (day 0) and after surgery (days 1-4)

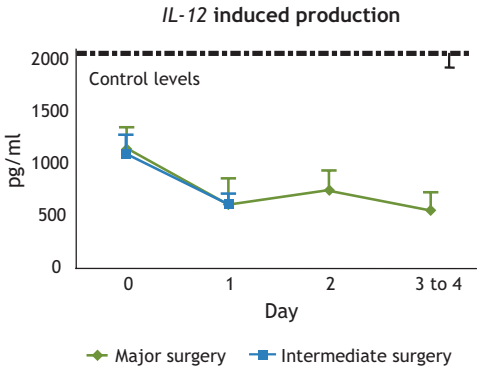



surgery. Measures included NK cytotoxicity, Th1 cytokine levels or their induced production, as well as various activation, adhesion, and recognition determinants of leukocytes (e.g., Figures 8 and 9).[21] The findings also suggest that neuroendocrine, rather than cytokine mediators, underlie postoperative suppression of cellular immunity.

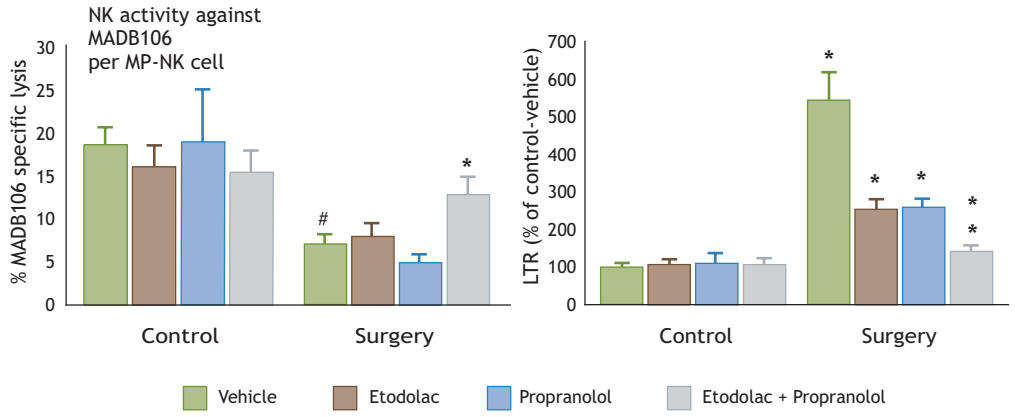
The following prophylactic approaches were developed to overcome the effects of stress and surgery (in rodents):

- We conducted spinal block in rats undergoing surgery, and were able to markedly reduce the tumor promoting effects of surgery (see Figure 3, p. 73).[11,22] We ascribe these benefits to the established ability of spinal block to reduce pain and various stress responses to surgery.
- We have successfully tested in rats and mice a drug regimen that could be used in cancer patients before, during, and after surgery. This regimen is based on simultaneous administration of the selective COX2 inhibitor, etodolac (prevents prostaglandin synthesis), and the β-adrenergic blocker, propranolol, both of which are routinely used clinically. These drugs reduced postoperative suppression of NK activity in a synergistic manner (Figure 10, p. 77), and abrogated the metastasis-promoting effects of surgery (Figure 11, p. 77) and of the removal of the primary tumor (Figure 12, p. 78).
- We developed immunostimulatory approaches based on repeated pre-surgical administration of low doses of either poly I-C[18,23], *IL-12*[24], or type-C CpG. These BRMs have most commonly been used in cancer patients long after surgery or in doses that had toxic effects. Our low dose regimens during the preoperative period increased host immune resistance to tumor progression, but did not prevent postoperative immune suppression. Therefore, the clinical perioperative use of such approaches is hampered by the expected immune suppression. Thus, we are currently integrating such preoperative immuno-stimulatory approaches with blockade of the immune-suppressive effects of surgery, employing selective COX2 inhibitors and β-adrenergic blockers. In an ongoing study we found such an integrated approach (*IL-12*, etodolac and propranolol) to boost cellular immunity and protect it from immune suppression, providing optimal protection against postoperative metastasis.

**FIGURE 9** *IL-12* induced production levels in matched controls and in patients before surgery (day 0) and after surgery (days 1-4)



**FIGURES 10 & 11** A synergistic impact of etodolac and propranolol in attenuating the NK-suppressive (Figure 10) and the metastasis promoting effects of surgery (Figure 11)




## Future Research Directions

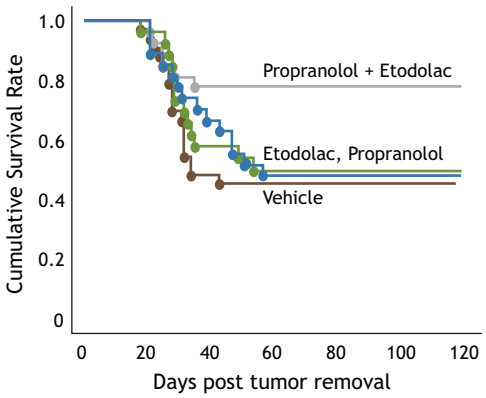
We aim at testing the above prophylactic approaches in cancer patients. Specifically, we are now in the process of initiating a preliminary randomized clinical trial to test our intervention based on etodolac and propranolol (COX2 inhibition and a-adrenergic blockade) administration before, during, and after surgery. The study is proposed in women undergoing breast cancer surgery, but similar studies could be conducted in other prevalent types of metastatic cancers. The measured outcomes will include perioperative levels of pro-angiogenic factors and neuroendocrine stress responses, the use of postoperative opiates, levels of cellular immune indices before and after surgery, and 5-year recurrence rates. The advantages of the above pharmacological intervention are its promising outcomes, minimal or no side effects, and relatively easy and safe use of established and inexpensive drugs. Both drugs, as well as similar drugs, have been used during surgery for other purposes. We expect our intervention to improve outcomes in all the above measures, and to suggest promotion of long-term survival rates, a finding that would have to be tested in a larger clinical trial.

We intend to continue our studies in animal models and to assess the impact of psychological stress on the efficacy of immunostimulatory regimens. This is a neglected field of research with potentially marked clinical ramifications, as most cancer patients are under psychological stress when receiving immunotherapy. These stress conditions have not been simulated in animal studies of cancer immunotherapy.

*We have successfully tested in rats and mice a drug regimen that could be used in cancer patients before, during, and after surgery.*

We will continue to develop approaches of integrated perioperative treatments based on preoperative immune stimulation and perioperative prevention of the impacts of psychological and surgical stress responses. We will study these approaches with respect to their postoperative immune protective

**FIGURE 12** Etodolac and propranolol synergistically improve survival rates following the removal of a primary metastasizing tumor.




characteristics, using various models of tumor progression. Such integrated approaches can prevent the impact of psychological stress on cellular immunity before surgery, improve the efficacy of immune stimulation, and protect immunity from suppression by surgery.

## Significant Results

Recent human studies support our hypotheses and the clinical feasibility and efficacy of our proposed interventions. Supporting the clinical feasibility and the potential benefits of our drug regimen described above is a recently published clinical *retrospective* study in breast cancer patients.[25] This study implemented an approach first presented by us in rats subjected to surgery, in which we added spinal block to general anesthesia.[11]

In the clinical study, women undergoing surgical excision of breast cancer that received a prostaglandin synthesis inhibitor during surgery and were subjected to spinal block of the sympathetic nerve system by paravertebral anesthesia (high-level regional block), exhibited a surprising four-fold reduction in cancer recurrence three years following surgery, compared to women who were not subjected to both treatments (6% vs. 24%, respectively).[25] These findings should be tested in a prospective study, and we plan to conduct a similar study employing our more-easily implemented pharmacological approach on prostaglandin and sympathetic blockade.

Additional support to our hypothesis that reducing perioperative suppression of cellular immunity in cancer patients will reduce postoperative cancer recurrence is based on current literature. In a recent review[1] we pointed out that the perioperative levels of NK activity are predictive of survival rates. Moreover, **modification in surgical procedures that enhanced or reduced immune suppression (e.g., anesthetic techniques, minimal invasive procedures, and blood transfusion), also respectively enhanced or reduced tumor recurrence.** Although correlative in nature, these associations remained significant after taking into account known prognostic factors. Some of these finding were achieved in randomized studies.

## Implications for Cancer Control

Although in cancer patients the immune system has failed to control the primary tumor, it is now clear that the immune system has massively interacted with the primary tumor along its evolvement.[1]

This is best indicated by the many specific immune escape mechanisms exhibited by most human cancers. It is also acknowledged that cellular immunity and other physiological mechanisms can limit the metastatic process while failing to control the primary tumor. Specifically, whereas following the excision of the primary tumor the great majority of cancer patients has single tumor cells in the circulation or bone marrow, most do not proceed to develop secondary cancer. This can be ascribed, at least partly, to immune surveillance of minimal residual disease (MRD).

Therefore, **maintaining potent cellular immune functioning during the perioperative period could be critical for ensuring long-term survival.** It is also crucial to recognize that the perioperative period, which is characterized by many risk factors for cancer progression (see "Perioperative Processes that Promote Tumor Progression" earlier in this section), also presents a short window of opportunity to eradicate cancer or ensure control of MRD. That is, once the major mass of dividing/mutating metastasizing malignant cells is eliminated, along with its established immune suppressive effects, the immune system needs only to control MRD. If it succeeds during the first few postoperative days by preventing the eruption of preexisting micrometastases and the seeding and establishment of newly released tumor cells, then the chances for long-term arrest of cancer progression increase markedly, as the many risk factors associated with tumor excision rapidly subside. Thus, our approach, which stresses the importance of achieving and maintaining potent cellular immunity during the perioperative period, may bear significant clinical ramifications potentially yielding increased long-term survival rates.

*In the clinical study, women undergoing surgical excision of breast cancer that received a prostaglandin synthesis inhibitor during surgery and were subjected to spinal block of the sympathetic nerve system by paravertebral anesthesia (high-level regional block) exhibited a surprising four-fold reduction in cancer recurrence three years following surgery, compared to women who were not subjected to both treatments.*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## Shamgar Ben-Eliyahu, PhD

Shamgar Ben-Eliyahu is a professor of Neuroimmunology and Psychoneuroimmunology (PNI) at Tel Aviv University, and a member of the Outcome Research Institute, Louisville, Kentucky. His main research interests are the impacts of surgery and stress (and the bioactive substances associated with them) on cell-mediated immunity (specifically NK cells) and on resistance to tumor progression. The current focus of his work is devising integrated approaches to reduce postoperative cancer recurrence through the use of immunological, physiological and psychological interventions during the immediate perioperative period. Among future research plans are clinical studies of prophylactic measures against the immunosuppressive and metastasis-promoting effects of surgery in cancer patients. Other interests include a) the impact of the menstrual cycle and sex hormones on stress responses, immunity, and tumor development; b) cellular immunity in schizophrenia and individuals with PTSD; c) the impact of maternal immune and stress responses on offspring neurodevelopment; and d) the role of stress and NK cells in the etiology of recurrent miscarriage.

Dr. Ben-Eliyahu conducts his studies employing *in vitro*, *ex vivo* and *in vivo* approaches, using immunological, physiological, and behavioral techniques; animal models of immunological and clinical relevance; and human subjects. Support for his studies has been provided by NCI/NIH, the Israeli Science Foundation, the Israel Ministry of Health, the Bi-national (Israel-USA) Science Foundation, and other sources.

Dr. Ben-Eliyahu's academic background is in Mathematics and Computer Sciences (B.Sc. at the Hebrew University of Jerusalem, Israel), Biological Psychology (M.A., & Ph.D. at UCLA, USA), and Neuroimmunology and Neuroendocrinology of Reproduction (post-doctorate training at the Department of Anatomy and the Neuro-Psychiatric Institute at UCLA). Dr. Ben-Eliyahu received several excellence awards along his studies, including the Young Investigator Award from the fund of PNI. Dr. Ben-Eliyahu took a tenure-track position in the Department of Psychology, Tel Aviv University in 1993, and became a full professor in 2003. He has served on the editorial board for several journals and is a reviewer for many others. He has also served on study sections for NIH, and reviewed grant applications for several national and international foundations. Dr. Ben-Eliyahu was invited to give multiple presentations at international forums. Last, Dr. Ben-Eliyahu is actively involved in teaching undergraduate and graduate students, and has received several awards for Outstanding Teaching. He is mentoring research students and advanced researchers in his lab, and is involved in scientific training of physicians.

NATURE REVIEWS

OPINION

# The influence of bio-behavioural factors on tumour biology: pathways and mechanisms

*Michael H. Antoni, Susan K. Lutgendorf, Steven W. Cole, Firdaus S. Dhabhar, Sandra E. Sephton, Paige Green McDonald, Michael Stefanek and Anil K. Sood*

Abstract | Epidemiological studies indicate that stress, chronic depression and lack of social support might serve as risk factors for cancer development and progression. Recent cellular and molecular studies have identified biological processes that could potentially mediate such effects. This review integrates clinical, cellular and molecular studies to provide a mechanistic understanding of the interface between biological and behavioural influences in cancer, and identifies novel behavioural or pharmacological interventions that might help improve cancer outcomes.

Clinical studies indicate that stress, chronic depression, social support and other psychological factors might influence cancer onset and progression[1–5]. Recent mechanistic studies have identified biological signalling pathways that could contribute to such effects. Environmental and psycho-social processes initiate a cascade of information-processing pathways in the central nervous system (CNS) and periphery, which subsequently trigger fight-or-flight stress responses in the autonomic nervous system (ANS), or defeat/withdrawal responses that are produced by the hypothalamic–pituitary–adrenal axis (HPA)[6]. FIGURE 1 shows the areas of the brain that are thought to be responsible for mediating stress responses and their effects on the adrenal glands and other target tissues. Cognitive and emotional feedback from cortical and limbic areas of the brain modulate the activity of hypothalamic and brain-stem structures that directly control HPA and ANS activity[7].

HPA responses are mediated by hypothalamic production of corticotrophin-releasing factor and arginine vasopressin, both of which activate the secretion of pituitary hormones such as adrenocorticotropic hormone (ACTH), enkephalins and endorphins. ACTH induces downstream release of glucocorticoids such as cortisol from the adrenal cortex. Glucocorticoids control growth, metabolism and immune function, and have a pivotal role in regulating basal function and stress reactivity across a wide variety of organ systems[8]. ANS responses to stress are mediated primarily by activation of the sympathetic nervous system (SNS) and subsequent release of catecholamines (principally noradrenaline and adrenaline) from sympathetic neurons and the adrenal medulla. Levels of catecholamines are increased in individuals who experience acute or chronic stress, and are responsible for ANS effects on cardiac, respiratory, vascular and other organ systems[8]. Examples of stressors associated with alterations in the HPA and/or ANS include marital disruption, bereavement, depression, chronic sleep disruption, severe trauma and post-traumatic stress disorder[9,10].

The activation of these pathways prepares an individual to survive a threat, and the physiological stress responses are therefore generally considered adaptive. However, under chronic stress most physiological systems are negatively affected by prolonged exposure to glucocorticoids and catecholamines[11]. These changes are manifested by deleterious health consequences such as increased risk for cardiac disease, slower wound healing and increased risk from infections[11]. In the past decade, it has become increasingly clear that chronic alterations in neuroendocrine dynamics can also alter multiple physiological processes involved in tumour pathogenesis[12–15].

In this article, we review the clinical and experimental evidence regarding the effects of stress on tumour development, growth and progression. Special emphasis is placed on neuroendocrine influences on the tumour microenvironment, viral oncogenesis and the immune system (FIG. 2). Although the mechanisms and clinical relevance of these pathways are described separately, there are numerous interactions between them, reflecting the complexity of cancer pathogenesis. These pathways might provide additional clues about factors that regulate the course of disease in cancer patients and might offer new opportunities for therapeutic interventions.

## Endocrine stress response and cancer

There is evidence linking stress, concomitant behavioural response patterns and resultant neurohormonal and neurotransmitter changes (all of which are referred to collectively within this paper as bio-behavioural factors) to cancer development and progression. Epidemiological data show that psychological and social characteristics might be associated with differential cancer onset, progression and mortality. For example, a twofold increase in breast cancer risk is evident after disruption of marriage owing to divorce, separation or death of a spouse[5]. Data from 3 eastern and midwestern states in the United States indicate that cancer risk increases after chronic depression that has lasted for at least 6 years[16]. A third study found that the combination of extreme stress and low social support was related to a ninefold increase in breast cancer incidence[4]. However, findings have been inconsistent. In general, stronger relationships have been observed between psycho-social factors and cancer progression than between psycho-social factors and cancer incidence (see REF. 3 for a discussion of the strengths and weaknesses of this literature). Data from patients with existing tumours show that cancer diagnosis and treatment cause substantial distress, and that those who tend toward depressive coping methods, such as hopelessness and helplessness, might experience accelerated disease progression[2]. By contrast, positive factors such as social support and optimism have predicted longer survival[17,18]. Additionally, there are important interactions between behavioural stress factors and health behaviours — including smoking, insomnia, alcohol abuse and obesity — that might have a further impact on cancer risk[19]. Recent experimental studies have begun to elucidate the mechanisms underlying these observations.

Animal models have provided compelling evidence regarding the effects of behavioural stress on tumorigenesis and the biological mechanisms involved (TABLE 1). For example, immobilization stress in rats that were given a carcinogen, diethylnitrosamine, increased both the

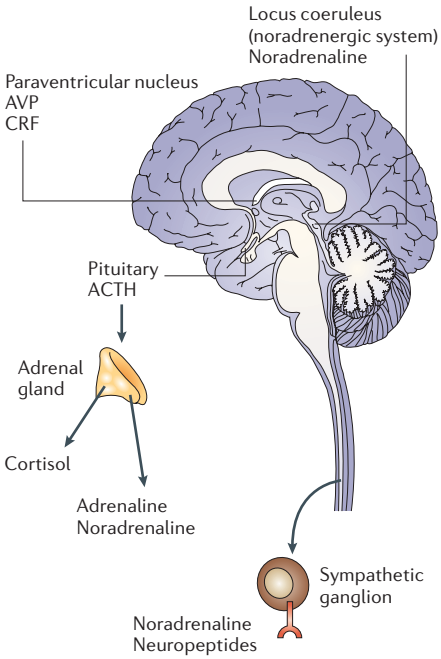


Figure 1 | **Important components of the central and peripheral stress systems.** Stressful experiences activate components of the limbic system, which includes the hypothalamus, the hippocampus, the amygdala, and other nearby areas. In response to neurosensory signals, the hypothalamus secretes corticotrophin-releasing factor (CRF) and arginine vasopressin (AVP), both of which activate the pituitary to produce hormones such as adrenocorticotropic hormone (ACTH). Circulating ACTH stimulates the production of glucocorticoids from the adrenal cortex. The sympathetic nervous system originates from the brainstem, and the pre-ganglionic neurons terminate in the ganglia near the spinal column. From these ganglia, post-ganglionic fibres run to the effector organs. The main neurotransmitter of the pre-ganglionic sympathetic fibres is acetylcholine and the typical neurotransmitter released by the post-ganglionic neurons is noradrenaline. The adrenal medulla contains chromaffin cells, which release mainly adrenaline.

incidence and rate of tumour growth[20]. Experimental stressors have also been found to increase the pathogenesis of various virally mediated tumours in animal models (see below). Swim stress, surgical stress, social confrontation and hypothermia resulted in increased lung metastasis from injected breast cancer cells[21–24]. Swim stress, laparotomy (opening the abdomen) and social confrontation caused a 2- to 5-fold increase in the number of rat MADB106 breast tumour metastases present in the lung[24,25] and a similar increase in the number of lung metastases counted 3 weeks later[24–26]. β-Adrenergic agonists (which simulate activation of the SNS) such as metaproterenol show dose-dependent increases in lung tumour metastases. Similarly, adrenaline injections promoted mammary tumour metastasis[21–24]. Perhaps most importantly, pre-treatment of animals with β-adrenergic antagonists (to block the activity of SNS activation) and indomethacin (to block inflammation) synergistically blocked the effects of behavioural stress on lung tumour metastasis[27].

Cellular and molecular events that promote cancer growth are also affected by stress. Swim stress in rodents results in induction of chromosomal aberrations and sister chromatid exchanges[28] as well as lower activity of metaphase nucleolar organizer regions in bone marrow cells[29]. These findings indicate that stress might compromise DNA repair mechanisms. Stress can also influence the expression of viral oncogenes and replication of tumorigenic viruses (see below). In an orthotopic murine model of ovarian carcinoma, immobilization stress increased tumour burden and enhanced angiogenesis and tumour production of vascular endothelial growth factor (VEGF)[30], indicating that stress might promote tumour growth by facilitating development of a blood supply. VEGF is a pro-angiogenic molecule that stimulates endothelial cell migration, proliferation and proteolytic activity[31]. VEGF also interferes with the development of T cells and the functional maturation of dendritic cells[32,33], indicating possible effects on anti-tumour immune responses (see below). In line with these findings, recent clinical studies have shown links between higher levels of social support and lower serum levels of VEGF in patients with ovarian cancer[34]. Furthermore, social support has also been linked to lower levels of interleukin-6 (IL-6), another pro-angiogenic factor, both in peripheral blood and in ascites from patients with ovarian cancer[35].

Understanding the mechanisms responsible for mediating the effects of stress on human tumour tissues is crucial for determining the full impact of stress on tumorigenesis and for devising effective interventions. Experimental evidence indicates that stress hormones have multiple effects on human tumour biology. Hormones that are associated with SNS activation might favour angiogenesis in human tumours. Noradrenaline has been shown to upregulate VEGF in adipose tissue and two ovarian cancer cell lines through the β-adrenergic receptor (βAR)–cyclic AMP (cAMP)–protein kinase A (PKA) pathway[36,37]. This effect was abolished by a β-blocker, propranolol, and was mimicked by isoproterenol (a synthetic drug that mimics the effects of SNS stimulation), and was therefore thought to be mediated through βARs[36,37]. Noradrenaline also promotes various steps that are essential to tumour metastasis, including invasion and migration. In *in vitro* experimental models, noradrenaline increased colon cancer cell migration, an effect that was inhibited by β-blockers[38]. Both adrenaline and noradrenaline promoted *in vitro* invasion of ovarian cancer cells by increasing the expression levels of matrix metalloproteinase 2 (MMP2) and MMP9 [12].

βARs, which mediate most of the effects of catecholamines, have been identified on breast and ovarian cancer cells[12,13]. In both of these studies, $\beta_2$AR was the dominant adrenergic receptor present. βARs are G-protein-coupled receptors whose primary function is the transmission of information from the extracellular environment to the interior of the cell, leading to activation of adenylyl cyclase and accumulation of the second messenger cAMP[39]. In mammary tumours, activation of βARs has been linked to accelerated tumour growth[13–15]. The cAMP-responsive-element-binding (CREB) protein is an important transcription factor that is activated by multiple signal-transduction pathways in response to external stimuli, including stress hormones[40,41]. Several studies have shown a role for the CREB family of proteins in tumour cell proliferation, migration, angiogenesis and inhibition of apoptosis[40–42], as well as the expression of viral oncogenes (see below). An additional cAMP target, EPAC (also known as Rap guanine-nucleotide-exchange factor 3 (RAPGEF3)) is an exchange protein that is directly activated by cAMP. EPAC was recently shown to control a number of cellular processes that were previously attributed to PKA[43]. For example, βAR-mediated activation of cAMP promotes ovarian cancer cell adhesion through the EPAC–RAP1 pathway[44]. Collectively, these studies demonstrate the growing evidence that mediators of SNS activate cellular pathways within tumours that contribute to growth and progression. However, the clinical relevance in human studies of the bio-behavioural stress mechanisms described above remains to be demonstrated.

### Glucocorticoids and other mediators

Glucocorticoids regulate a wide variety of cellular processes through glucocorticoid-receptor-mediated activation or repression of target genes. Recent studies have demonstrated that whereas glucocorticoid hormones induce apoptosis in lymphocytes[45],

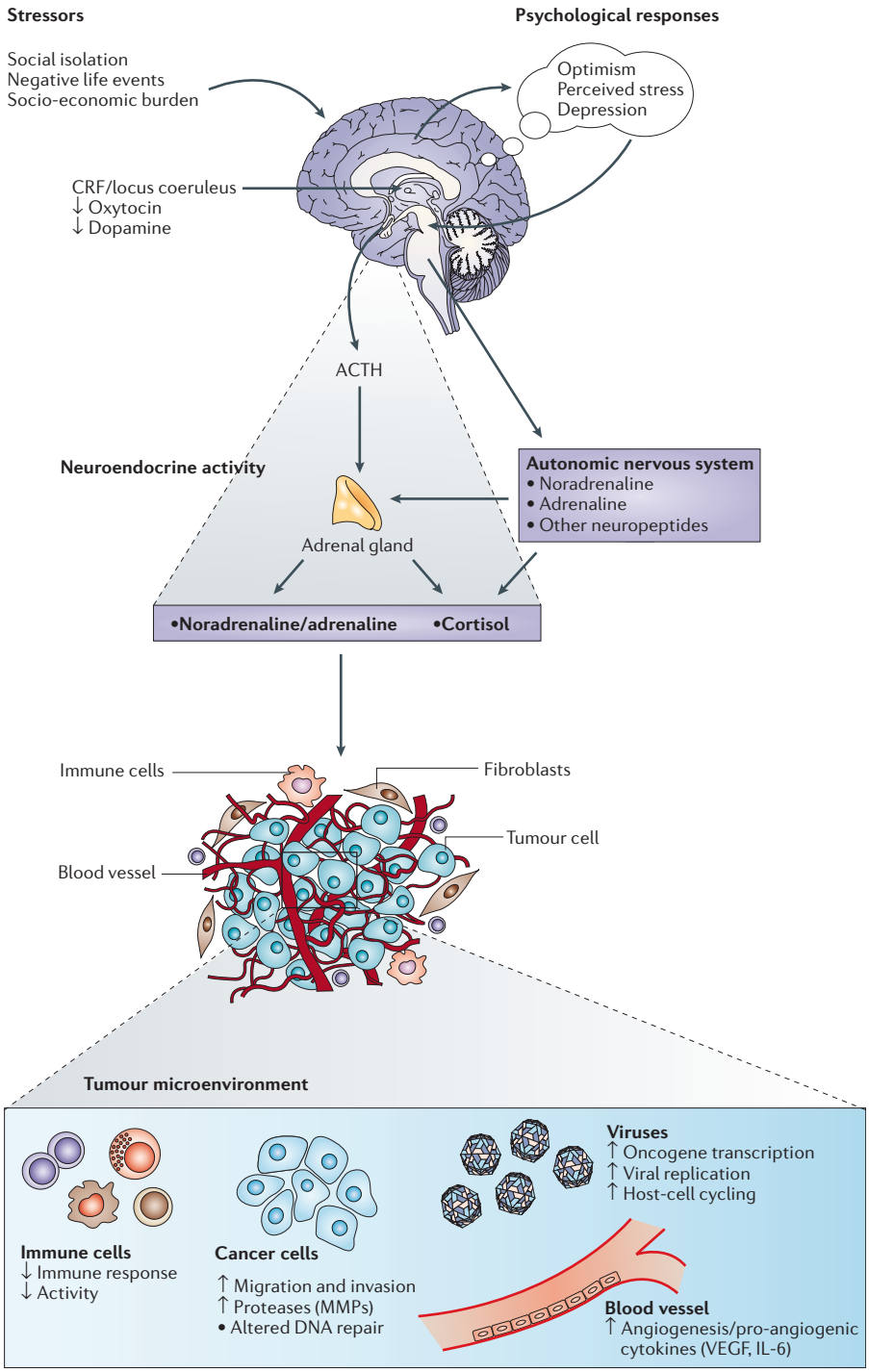


Figure 2 | **Effects of stress-associated factors on the tumour microenvironment.** The responses to stressors involve central nervous system (CNS) perceptions of threat and subsequent activation of the autonomic nervous system (ANS) and the hypothalamic–pituitary–adrenal (HPA) axis. Catecholamines, glucocorticoids and other stress hormones are subsequently released from the adrenal gland, brain and sympathetic nerve terminals and can modulate the activity of multiple components of the tumour microenvironment. Effects include the promotion of tumour-cell growth, migration and invasive capacity, and stimulation of angiogenesis by inducing production of pro-angiogenic cytokines. Stress hormones can also activate oncogenic viruses and alter several aspects of immune function, including antibody production, cytokine production profiles and cell trafficking (see REF. 6 for a comprehensive review of immune effects). Collectively, these downstream effects create a permissive environment for tumour initiation, growth and progression. CRF, corticotrophin-releasing factor; IL-6, interleukin-6; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor.

these hormones activate survival genes that protect cancer cells from the effects of chemotherapy in both *in vitro* and *in vivo* experimental models[46,47]. Glucocorticoids can also activate oncogenic viruses and inhibit anti-tumour and antiviral cellular immune responses (see below). Glucocorticoids such as cortisol might function in a synergistic fashion with catecholamines to facilitate cancer growth. For example, in lung carcinoma cells cortisol increased βAR density and potentiated the isoproterenol-induced increase in cAMP accumulation[48]. So, it is plausible that stressful situations characterized by both increased catecholamine and cortisol concentrations (for example, uncontrollable stress) might have the greatest impact on cancer-related processes.

The expression levels of other hormones affected by stress include prolactin, which increases with stress[49,50], and oxytocin and dopamine, which decrease with stress[51]. Prolactin can promote cell growth and survival in breast tumour and other tumour cells[52]. Oxytocin inhibits the growth of epithelial cell (such as breast and endometrial) tumours and those of neuronal or bone origin, but the hormone has a growth-stimulating effect in trophoblast and endothelium tumours[53]. For example, exogenous oxytocin has a dose-dependent mitogenic effect on human small-cell lung cancer cell lines, which is blocked by an oxytocin receptor antagonist[54]. Dopamine, which is known to inhibit the growth of several types of malignant tumours[55], blocks VEGF-induced angiogenesis both *in vitro* and *in vivo*, primarily by inducing endocytosis of VEGF receptor 2 in endothelial cells[56].

### Effect of circadian deregulation on cancer

Evidence indicates that circadian deregulation influences the secretion of some stress-associated hormones, and this might explain the associations between stress and cancer[57,58]. Data from separate lines of investigation show that stress can disrupt circadian glucocorticoid rhythms[57,59] and favour tumour initiation and progression[57,58,60]. Night-time shift work, a condition that is known to disrupt endocrine rhythms, is a risk factor for breast and colorectal cancer[61]. Mice with circadian disruption owing to *Per1* (period 1) or *Per2* gene mutations are prone to tumour development and early death[62,63]. Tumour-bearing animals and cancer patients have disrupted endocrine, metabolic and immunological cycles, with greater disruption in cases where the tumour is advanced or fast-growing[64]. In murine studies, tumour progression and mortality are dramatically

Table 1 | **Effects of stress and stress-associated hormones on cancer**

| Experimental manipulation | Animal | Biological effect | Tumour type | Effect on tumour growth | References |
|---|---|---|---|---|---|
| Confrontation | Rats | NA | Breast | Increased metastasis of tumour cells to the lung | 25 |
| Restraint stress | Rats | Decreased numbers of T cells | Mammary | Increased growth during stress | 144 |
| Forced swim | Rats | Decreased natural-killer-cell activity | Leukaemia | Increased mortality | 22 |
| Abdominal surgery | Rats | Decreased natural-killer-cell activity | Mammary | Increased metastasis of tumour cells to the lung | 22 |
| High versus low dopaminergic reactivity | Rats | Decreased angiogenesis with high dopaminergic reactivity | Mammary | Fewer lung metastasis with increased dopaminergic reactivity | 145 |
| Dopamine administration | Mice | Decreased angiogenesis; decreased VEGF–VEGFR2 binding and phosphorylation | Ovarian | Decreased ascites formation | 56 |
| Dopamine administration | Mice | Decreased angiogenesis | Gastric | Decreased growth | 55 |
| Social isolation | Mice | Decreased macrophage activity | Ehrlich | Increased growth | 146 |
| Immobilization stress | Mice | Increased angiogenesis | Ovarian | Increased growth | 30 |
| Restraint stress | Mice | Decreased IL-12, IFNγ, CCL27 (also known as CTACK) and numbers of infiltrating T cells; increased numbers of suppressor cells | Skin and squamous cell carcinoma | Increased incidence, number, size and density | 110 |

CTACK, cutaneous T-cell attracting chemokine; IL-12, interleukin-12; IFNγ, interferon-γ; NA, not available; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2.

accelerated after elimination of circadian rhythms by manipulation of light–dark cycles (imposed 'jet-lag') and by the use of bilateral electrolytic lesions to destroy the suprachiasmatic nuclei (SCN), which eliminates circadian rhythms[60]. Two clinical studies have also shown that the status of circadian cycles, such as cortisol or the 24-hour-rest–activity cycle, can predict long-term cancer survival[58,65].

Stress-related disruption of circadian cycles might impair cancer-defence mechanisms through genetic and/or glucocorticoid and immune pathways. Animal studies show that behavioural factors such as imposed chronic jet-lag can alter *Per1* expression in the SCN[60], and circadian genes, including *Per1*, regulate tumour suppression, cellular response to DNA damage, and apoptosis[63]. Glucocorticoid rhythms that are driven by the SCN[62] are linked to both enumerative and functional immunity[66]. Sleep disruption can increase the release of cortisol as well as increase the expression of pro-inflammatory cytokines (for example, IL-6 and tumour-necrosis factor-α (TNFα)) in cancer patients[67]. Pro-inflammatory cytokines might promote tumorigenesis by inducing DNA damage or inhibiting DNA repair through the generation of reactive oxygen species. Pro-inflammatory cytokines can also lead to the inactivation of tumour-suppressor genes, the promotion of autocrine or paracrine growth and survival of tumour cells, the stimulation of angiogenesis, or the subversion of the immune response (which leads to the activation of B cells rather than T cells in the tumour microenvironment)[68]. Conversely, agents that are capable of re-establishing circadian regulation (for example, melatonin) might have anti-tumour effects. Research on oestrogen-receptor-positive MCF-7 human breast cancer cells has shown that melatonin reversibly inhibits cell proliferation, increases p53 expression, modulates the cell cycle, and reduces metastatic capacity by increasing the expression of cell-surface adhesion proteins[69,70]. Taken together, these data indicate a potentially important role of circadian regulation in cancer defence and treatment[62].

### Influences on viral oncogenesis

The first experimental demonstration that bio-behavioural factors could promote cancer came from animal studies of tumour viruses[71]. Many studies have demonstrated the accelerated growth of virally induced tumours in stressed animals, as well as the more surprising protective effects of handling, fighting and crowding[72,73]. Neuroendocrine function has a central role in these processes because it can modulate viral replication, activate viral oncogenes, increase tumour metabolism and regulate the immune response[74–76]. The evidence for a viral contribution to human cancer has grown[77] (BOX 1), and stress hormones have

Box 1 | **Physiological pathways, bio-behavioural processes and oncogenesis**

- Environmental and social processes activate interpretive processes in the central nervous system (CNS) that can subsequently trigger fight-or-flight stress responses in the autonomic nervous system (ANS) or defeat/withdrawal responses through the activation of the hypothalamic–pituitary–adrenal axis (HPA)[141].
- Individual differences in perception and evaluation of external events (coping) creates variability in individual ANS and HPA activity levels.
- Over long periods of time, these neuroendocrine dynamics can alter various physiological processes involved in tumorigenesis, including oxidative metabolism, DNA repair, oncogene expression by viruses and somatic cells, and production of growth factors and other regulators of cell growth.
- Once a tumour is initiated, neuroendocrine factors can also regulate the activity of proteases, angiogenic factors, chemokines and adhesion molecules involved in invasion, metastasis and other aspects of tumour progression.
- CNS processes can also shape behavioural processes that govern cancer risk (for example, smoking, transmission of oncogenic viruses or exposure to genotoxic compounds).

Box 2 | **Viral oncology**

- Viral infections contribute to approximately 15% of human cancers worldwide[77].
- Pathogenic mechanisms include expression of viral oncogenes (for example, human T-cell lymphotropic virus Tax, and Epstein–Barr virus nuclear antigens and latent membrane protein 1), inhibition of host-cell tumour-suppressors (for example, human papillomavirus E6, which targets p53 and E7, which targets RB), and genomic damage stemming from immune-mediated cell turnover (for example, hepatitis B and C viruses)[77,142,143].
- All major human tumour viruses are sensitive to the intracellular signalling pathways activated by the hypothalamic–pituitary–adrenal axis and autonomic nervous system. These mediators can reactivate latent tumour viruses, stimulate oncogene expression and inhibit host-cell antiviral responses.

been found to influence the activity of various human tumour viruses (BOX 2; TABLE 2).

Epstein–Barr virus (EBV) is reactivated in healthy people who experience prolonged psychological stress[78,79]. In these studies HPA activity increased in parallel with reactivation of EBV[79,80], and glucocorticoid hormones were subsequently found to increase EBV gene expression *in vitro*[80,81]. High-risk human papilloma viruses (HPVs), which contribute to cervical and rectal carcinomas, also respond to glucocorticoids by activating gene expression[82–84], interacting with cellular proto-oncogenes such as *HRAS*[85], and evading cellular immune responses by downregulating the expression of tumour MHC-I (major histocompatibility complex class I) molecules[86]. Clinical studies have identified stressful life events as a risk factor for increased progression of cervical dysplasia in HPV-positive women[87,88]. Furthermore, glucocorticoid antagonists can inhibit HPV activity *in vitro*[89–91], providing a molecular rationale for clinical interventions that target HPA activity. Although hepatitis B and C viruses come from different viral lineages, glucocorticoids increase gene expression in and replication of both viruses[90,92–94]. These dynamics are so pronounced that glucocorticoids are employed clinically to activate hepatitis B and C viruses for eradication by replication-dependent antiviral drugs[93,95].

Cancer-related viruses are also sensitive to catecholamines and the PKA signalling pathway. Molecular mechanisms are especially well defined for AIDS-associated malignancies. Catecholamines can accelerate human immunodeficiency virus 1 (HIV1) replication by increasing cellular susceptibility to infection[96,97], activating viral gene transcription[96] and suppressing antiviral cytokines[98]. People with heightened ANS activity show an increased viral load in the plasma and an impaired response to antiretroviral therapy[96], placing them at increased risk for AIDS-associated B-cell lymphomas[99]. Catecholamines can also activate the Kaposi sarcoma-associated herpesvirus (KSHV) through PKA induction of the viral transcription factor Rta[100]. Human T-cell lymphotropic viruses 1 and 2 (HTLV1 and HTLV2, respectively) are sensitive to PKA-mediated induction of the oncogenic Tax transcription factor[101]. Hormonal regulation of viral replication represents an important pathway by which bio-behavioural factors might influence malignant processes, but it also indicates novel therapeutic approaches such as β-adrenergic priming of viral genomes for clearance by replication-dependent nucleoside analogue drugs.

In addition to direct effects on viral gene expression, bio-behavioural factors can also indirectly affect tumour viruses by modulating host immune responses (see below). Antiviral vaccines will have an increasing role in the primary prevention of virally mediated cancers, and bio-behavioural influences on vaccine-induced immune responses will become especially relevant[102,103]. Neuroendocrine influences on the immune response might also explain why oncogenic viruses so consistently acquire hormone-responsive replication dynamics. Viruses that coordinate their gene expression with periods of hormone-induced immunosuppression should enjoy a significant survival advantage. Similar selective pressures might also shape the evolution of non-viral malignancies[104] such that genomic alterations are selected based on their ability to evade immune clearance or to synergize with endocrine dynamics to optimize tumour growth and metastasis.

### Influences on immune mechanisms

Chronic stress has been shown to suppress different facets of immune function[2] such as antigen presentation, T-cell proliferation, and humoral and cell-mediated immunity, mainly through the release of catecholamine and/or glucocorticoid hormones[105–107]. Relevant neuroendocrine and immune system interactions include direct synapse-like connections between sympathetic nerves and lymphocytes in lymphoid organs[108], neural and endocrine modulation of lymphocyte trafficking[109], and modulation of leukocyte function through glucocorticoid receptors and other receptors[70]. Tumour incidence and progression based on modulation of the immune response by chronic stress has been demonstrated in many animal models (see above). Recent studies have shown that chronic stress experienced during exposure to non-blistering ultraviolet radiation significantly increases susceptibility to squamous cell carcinoma by suppressing type 1 cytokines and the infiltration of protective T cells. Regulatory or suppressor T-cell numbers within the tumours and in the circulation were also increased[110]. Studies in mice of the immune response to transplanted syngeneic tumours showed that noradrenaline[111] and adrenaline[112,113] directly inhibited the generation of anti-tumour cytotoxic T cells through β-adrenergic signalling mechanisms. Chronic stress has been shown to modulate lymphocyte apoptosis through

Table 2 | **Neuroendocrine influences on tumour viruses**

| Human tumour virus | Malignancy | Sensitivity* |
|---|---|---|
| Human papilloma viruses 16 and 33 | Cervical and head/neck cancer | HPA |
| Hepatitis B virus | Hepatocellular carcinoma | HPA |
| Hepatitis C virus | Hepatocellular carcinoma | HPA |
| Epstein–Barr virus | Lymphoma, and nasopharygeal carcinoma | HPA |
| Human T-cell lymphotropic viruses 1 and 2 | Adult T-cell leukaemia/lymphoma | ANS |
| Kaposi sarcoma-associated herpesvirus | Kaposi sarcoma, and primary effusion lymphoma | ANS |

*Presumptive, based on *in vitro* studies. ANS, autonomic nervous system; HPA, hypothalamic–pituitary–adrenal axis. Vaccination is an important primary prevention strategy against viral tumours, and behavioural factors can influence the efficacy of this approach by modulating vaccine-induced immune responses[102,103].

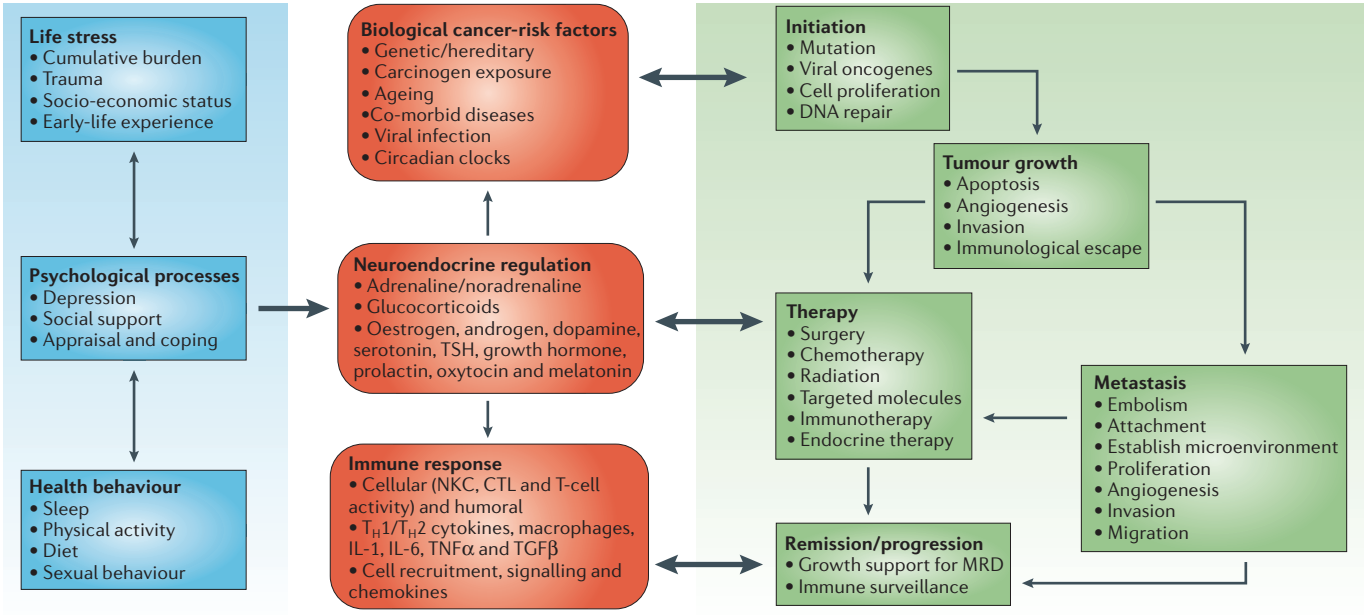


Figure 3 | **Integrated model of bio-behavioural influences on cancer pathogenesis through neuroendocrine pathways.** In this model, bio-behavioural factors such as life stress, psychological processes and health behaviours (blue panel) influence tumour-related processes (green panel) through the neuroendocrine regulation of hormones, including adrenaline, noradrenaline and glucocorticoids (red panel). Central control of peripheral endocrine function also allows social, environmental and behavioural processes to interact with biological risk factors such as genetic background, carcinogens and viral infections to systemically modulate malignant potential (red panel). Direct pathways of influence include effects of catecholamines and glucocorticoids on tumour-cell expression of genes that control cell proliferation, invasion, angiogenesis, metastasis and immune evasion (green panel). Stress-responsive neuroendocrine mediators can also influence malignant potential indirectly through their effects on oncogenic viruses and the cellular immune system (red panel). These pleiotropic hormonal influences induce a mutually reinforcing system of cellular signals that collectively support the initiation and progression of malignant cell growth (green panel). Furthermore, neuroendocrine deregulation can influence the response to conventional therapies such as surgery, chemotherapy and immunotherapy (green panel). In addition to explaining bio-behavioural risk factors for cancer, this model suggests novel targets for pharmacological or behavioural intervention. CTL, cytotoxic T lymphocytes; IL, interleukin; MRD, minimal residual disease; NKC, natural killer cell; TGFβ, transforming growth factor-β; TNFα, tumour-necrosis factor-α; TSH, thyroid-stimulating hormone.

an increase in FAS (also known as CD95 or APO1) expression. It has been hypothesized that such lymphocyte reduction might result in an increase in the incidence of oncogenic viral infections and DNA damage[114].

Compromised natural killer (NK)-cell function has been shown in both animal and clinical studies of surgical stress[22,115]. High levels of psychological distress have been linked to reduced cellular immunity in patients with breast[116] and ovarian cancer[117]. More specifically, distress measured by self-report was correlated with low NK-cell cytotoxicity in tumour-infiltrating lymphocytes from human ovarian cancers[117]. Low peripheral NK-cell counts are prognostic for early breast cancer mortality, and reduced NK-cell cytotoxicity is predictive of a poor clinical outcome in patients with breast carcinoma[58]. Positive psycho-social factors such as social support have been associated with increased levels of NK-cell cytotoxicity in patients with breast[118] and ovarian cancer[117]. The relationship of increased NK-cell cytotoxicity with social support was not limited to the periphery; it was also seen in tumour-infiltrating lymphocytes isolated from human ovarian cancers, reflecting possible psycho-social influences on the tumour microenvironment[117]. Patients with breast cancer who reported increased psychological growth through participation in a cognitive behavioural intervention programme demonstrated increased levels of cellular immune function[119]. Preliminary studies have found that the expression of spirituality was related to increased numbers of circulating T cells in patients with breast cancer[120], and that the use of humour as a coping mechanism was associated with increased NK-cell activity in cancer patients[121].

## Clinical opportunities and challenges

Our understanding of the biological and clinical significance of psycho-social and bio-behavioural influences on cancer pathogenesis is expanding. As described in this review, factors such as chronic stress, depression and social support have been linked to tumour biology, viral oncogenesis and cell-mediated immunity (FIG. 3). Although the molecular pathways have not been completely delineated, observations to date indicate a need for novel therapeutic paradigms that integrate a bio-behavioural perspective.

It is plausible that successful management of factors such as stress and negative mood might have a salubrious effect on the neuroendocrine regulation of oncogenesis, tumour growth and metastasis, and cancer immunoediting processes. Psycho-social interventions such as relaxation and cognitive behavioural techniques that alter negative mood seem to modulate ANS and HPA hormonal activity[122–124]. Moreover, such interventions can potentially be used in conjunction with conventional therapies to maximize treatment efficacy[125,126]. Stress-management interventions that dampen chronic-stress-related physiological changes might facilitate immune system 'recovery' and thereby increase immune surveillance during the active treatment of cancer[119,124]. Group-based psycho-social interventions that combine relaxation with cognitive behavioural techniques, such as cognitive behavioural stress management (CBSM), have been shown to increase indicators

of immune responses against potentially oncogenic viral infections, such as EBV[127]. Such alterations are paralleled by decreased expression levels of cortisol in the serum, a reduced depressive mood, increased social support and enhanced relaxation skills[122].

In HIV-infected individuals, who as a group are at risk for multiple opportunistic cancers, CBSM seems to accelerate reconstitution of naive T-lymphocytes, increase $CD8^+$ cytotoxic T-cell numbers and decrease the viral load of HIV over time[122,128]. These changes are pre-dated by decreases in negative mood and decreases in urinary cortisol and noradrenaline output[122,129]. It is plausible that CBSM might also help decrease the replication and function of other oncogenic viruses such as HPV and improve immune defences against them. Psycho-social interventions in cancer patients have resulted in alterations in neuroendocrine regulation and immunological functions[124,130,131] that are relevant for monitoring neoplastic cell changes. For example, two recent randomized clinical trials have documented increases in lymphocyte proliferation in patients with breast cancer following psycho-social interventions[119,124], and post-intervention changes in NK-cell activity have also been shown in patients with malignant melanoma[131]. Collectively, this work indicates that stress management can modify neuroendocrine deregulation and immunological functions that potentially have implications for tumour progression. This might be particularly important among vulnerable populations such as older adults because ageing is associated with a suppression of the immune response.

Clinical studies of psycho-social interventions with cancer survival as an outcome have been methodologically flawed or have failed to confirm a survival advantage in the treatment group[1,126,132–134]. Similar to most medical interventions for cancer, the effectiveness of psycho-social interventions is likely to vary with the type and stage of cancer, characteristics of the patient (for example, age, gender, education, co-morbid medical conditions, and health behaviours such as tobacco use, alcohol consumption and physical activity) and the type and delivery of the intervention. Nevertheless, epidemiological evidence correlating psychological and social factors (for example, chronic depression, hopelessness, marital disruption and social support) with cancer incidence, progression and survival give credence to examining the biological signalling pathways and mechanisms that underlie these observations.

Pharmacological interventions can potentially be used to ameliorate stress-associated influences on cancer development and progression. As discussed above, β-blockers have been shown to block many of the deleterious effects of stress. In a large case–control study of patients with prostate cancer who were taking anti-hypertensive medication, only β-blockers were associated with a reduction of cancer risk[135]. A cohort study of cardiovascular patients showed that the use of β-blockers, relative to never-using, resulted in a 49% decrease in cancer risk, with a 6% decrease in risk for every year of use[136]. Large population-based case–control studies have not confirmed alterations in risk for invasive breast carcinoma with β-blocker use[137,138]. The use of antidepressant medications might be promising, owing to a concomitant suppression of an inflammatory response that has been associated with certain types of cancer[139]. For example, lithium inhibits prostaglandin E1, and tricyclic antidepressants antagonize thromboxanes[140]. Some monoamine oxidase inhibitors exert a more potent anti-prostaglandin effect than indomethacin[140]. Whether these agents can be used to reduce cancer risk through bio-behavioural-related mechanisms remains to be determined, but these studies indicate that further inquiry is warranted.

## Conclusion

Despite significant progress in the past decade, further research is needed to define the mechanisms underlying the complex circuits involving the HPA and ANS axes and their effects on the processes involved in cancer development and progression. The body of data outlined above supports a model in which bio-behavioural factors influence multiple aspects of tumorigenesis through their impact on neuroendocrine function (FIG. 3). These effects include direct promotion of tumour growth by affecting steps in the metastatic cascade and viral oncogenesis. Furthermore, the interplay between behavioural processes and cellular immune factors also supports a favourable physiological environment for tumour establishment and growth. In the context of this 'systems biology' perspective, pharmacological and behavioural interventions that address neuroendocrine dysfunction could have a clinically significant role in avoiding these deleterious effects on tumour growth. Although stress *per se* does not cause cancer, the clinical and experimental data outlined above indicate that factors such as mood, coping mechanisms and social support can significantly influence the underlying cellular and molecular processes that facilitate malignant cell growth. As cancer treatment evolves towards a more patient-specific approach, consideration of the influence of bio-behavioural factors provides a novel perspective for mechanistic studies and new therapeutic targets.

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### Competing interests statement

The authors declare no competing financial interests.

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# Biological Mechanisms of Psychosocial Effects on Disease (BiMPED)

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The Biological Mechanisms of Psychosocial Effects on Disease (BiMPED) is a programmatic framework to cultivate the discovery of biological pathways that mediate influences of biobehavioral[a] factors on malignant growth. Animal models provide compelling evidence of behavioral stress and other influences on tumorigenesis that are mediated by the central nervous system. Additionally, clinical studies suggest associations between biobehavioral states such as chronic stress and depression, and variations in the progression of established tumors.

The Basic and Biobehavioral Research Branch (BBRB) seeks to encourage mechanistic studies to identify biological signaling pathways that might inform such observations. Our intent is to evaluate and encourage research that explores how neurotransmitters and neuropeptides associated with biobehavioral factors influence tumor processes like angiogenesis, apoptosis, invasion, inflammation, and metastasis.

*Psychoneuroimmunology (PNI) is the study of the interaction of behavioral, neural, and endocrine factors and the functioning of the immune system.*

BiMPED strives to support transdisciplinary research that bridges basic cancer biology and biobehavioral science to advance our fundamental knowledge of the extent and specificity by which central nervous system regulated factors like stress, chronic depression, and social support might regulate tumor biology. This perspective is based on the fundamental premise that any causal influence on cancer pathogenesis must ultimately be mediated by changes in the function of tumor cells, their micro- and macro-environment, or their antecedents (activity of tumor inducing viruses or mutagens, failure of DNA repair, epigenetic changes).

## History of BiMPED as a Programmatic Framework

The initial goal of BiMPED was to evaluate the applicability of psychoneuroimmunology[b] (PNI) research to cancer control. PNI research had made substantial contributions to our understanding of stress and immunity in HIV/AIDS, wound healing, and other immunologically mediated disease processes. Historically, this paradigm yielded limited significance for cancer control.

BiMPED began with a small meeting of NIH extramural program officers who shared a programmatic interest in PNI.

[a] Term used to describe interrelationships among psychosocial, behavioral, and biological processes, as in the progression or treatment of a disease.

[b] The study of the interaction of behavioral, neural, and endocrine factors and the functioning of the immune system.

Present at this initial meeting were representatives from the BBRB, the Office of Behavioral and Social Sciences Research (OBSSR), the National Institute of Mental Health (NIMH), the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute of Allergy and Infectious Diseases, and the NCI's Office of Cancer Complementary and Alternative Medicine (OCCAM).

Discussions from this initial NIH meeting were the impetus for the first scientific meeting of the initiative held in March 2002 (Figure 1). Goals for the meeting were to review current knowledge of biological mechanisms associated with psychosocial effects on disease, discuss the state of science and applicability of PNI-related research to cancer control, and identify critical research needs. Scientists from diverse behavioral and biomedical disciplines conducting both human and animal work in PNI and related fields presented research in their respective areas of expertise.

**FIGURE 1** BiMPED meeting, March 2002

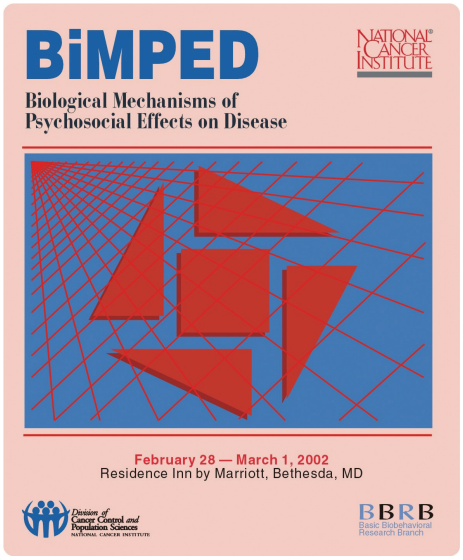


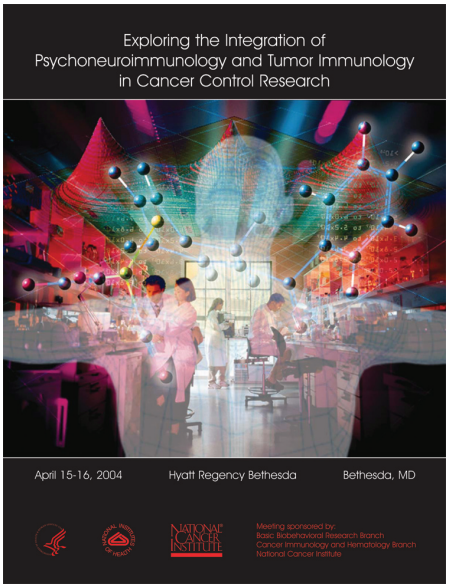

**FIGURE 2** Special issue of *Brain, Behavior, and Immunity*


A supplemental special issue of *Brain, Behavior, and Immunity* (February 2003, Volume 17, Supplement 1; Figure 2), the official journal of the Psychoneuroimmunology Research Society (PNIRS), was published to disseminate and expand the scientific discourse of the 2002 BiMPED meeting. The articles discussed research on interactions among behavior, neural, and endocrine function and immune system processes and links to health and disease. Each article addressed implications for cancer control and the supplement included cancer specific commentaries. The special issue was made possible by contributions from OCCAM, NIMH, NIAMS, and OBSSR.

In 2004, BBRB and the Cancer Immunology and Hematology Branch of the Division of Cancer Biology sponsored a meeting of PNI, tumor immunology, and cancer biology scientists to encourage the exchange of scientific ideas among the disciplines.

The goals of "Exploring the Integration of Psychoneuroimmunology and Tumor Immunology in Cancer Control Research" were to cultivate a common base of understanding between PNI scientists and tumor immunologists and biologists, and to discuss challenges to the advancement and appreciation of cancer-related PNI in cancer research (Figure 3). Clinical associations between biobehavioral factors and cancer progression were presented. It was determined that data to support these associations at the molecular level were scarce and a better understanding of the underlying biology remained critical.

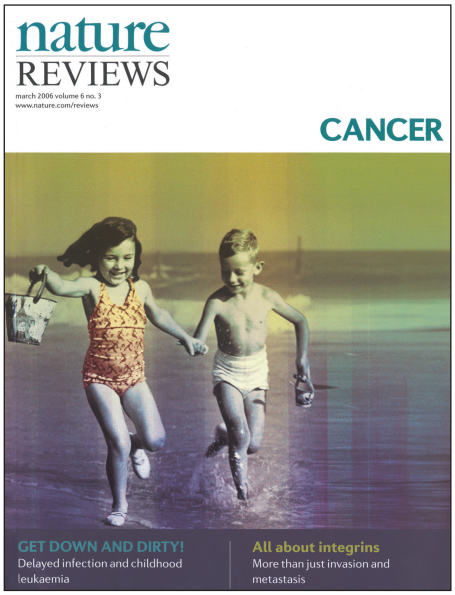
**FIGURE 3** The Psychoneuroimmunology Conference, April 2004




Historically, PNI research in cancer has focused on enumerative and functional assays of natural killer cells. There is a need to examine other immunological parameters and determine molecular mechanisms (T-cell activity, receptor function, signal transduction pathways) that explain these associations and why a particular molecular event is affected. Development of molecular models will help determine points during cancer progression in which intervention (pharmacologic or behavioral) would have the most promising effects.

The BiMPED initiative continues to be shaped by considerable input from our scientific constituency. BBRB has sponsored scientific programming at academic and research society meetings to present states of the science and to facilitate discussions of future research directions. BBRB has engaged extramural scientists to identify opportunities and challenges to our goal of accelerating progress in mechanistic studies of biobehavioral influences on tumor processes. With such scientists, we recently published a perspective in *Nature Reviews Cancer* (Antoni et al., 2006; see the "Nature Reviews" section in this book) that reviewed the clinical, epidemiological, and experimental evidence regarding the effects of stress and other psychosocial factors on tumor development, growth, and progression. This seminal publication proposed an integrated model of biobehavioral influences on cancer pathogenesis through neuroendocrine pathways (Figure 4).

**FIGURE 4** *Nature Reviews Cancer*



*BBRB has engaged extramural scientists to identify opportunities and challenges to our goal of accelerating progress in mechanistic studies of biobehavioral influences on tumor processes.*

### Extramural Portfolio

As reflected in our portfolio, BIMPED has evolved from an initiative to evaluate the applicability of PNI to cancer control to a research program that seeks to elucidate biological and molecular mechanisms associated with biobehavioral influences on cancer processes. We continue to cultivate a promising portfolio of research that spans the cancer control continuum from prevention to survivorship. Examples of funded research (R03, R21, and R01) from the BBRB portfolio include:

- **Fatigue, sleep and circadian rhythms in breast cancer**
  Sonia A. Ancoli-Israel
  University of California, San Diego
- **Contributions of sleep/rhythms/ fatigue to "chemobrain"**
  Sonia A. Ancoli-Israel
  University of California, San Diego
- **Psychological interventions for women with breast cancer**
  Barbara L. Andersen
  Ohio State University
- **Biobehavioral effects of emotional expression in cancer**
  Lorenzo Cohen
  University of Texas
  M.D. Anderson Cancer Center

- **Stress and UV-induced squamous cell carcinoma**
  Firdaus Dhabhar
  Stanford University
- **Effects of opioids on sleep and fatigue**
  Joel E. Dimsdale
  University of California, San Diego
- **Biobehavioral pathways in oral precancers**
  Carolyn Y. Fang
  Fox Chase Cancer Center
- **Psychological influences on immune responses to HPV**
  Carolyn Y. Fang
  Fox Chase Cancer Center
- **Cognitive functioning after breast cancer treatment**
  Patricia Ganz
  University of California, Los Angeles
- **Stress, the immune system, and basal cell carcinoma**
  Ronald Glaser
  Ohio State University
- **Tai chi effects on chronic insomnia in breast cancer survivors: Immune mechanisms**
  Michael Irwin
  University of California, Los Angeles
- **Biobehavioral-cytokine interactions in ovarian cancer**
  Susan K. Lutgendorf
  University of Iowa
- **Healing touch, immunity, and fatigue in breast cancer**
  Susan K. Lutgendorf
  University of Iowa
- **Cranial stimulation for chemo symptoms in breast cancer**
  Debra E. Lyon
  Virginia Commonwealth University
- **Immune dsyregulation by psychosocial distress**
  Herbert L. Mathews
  Loyola University, Chicago
- **PNI-based stress management in early breast cancer**
  Nancy L. McCain
  Virginia Commonwealth University
- **Psychological stress, cortisol, and B lymphocyte decrements**
  Bonnie A. McGregor
  Fred Hutchinson Cancer Research Center
- **PNI relations among women with endometrial cancer during the perioperative period**
  Deidre B. Pereira
  University of Florida
- **Ovarian cancer: Mechanisms of neuroendocrine regulation**
  Anil K. Sood
  University of Texas
  M.D. Anderson Cancer Center
- **Tumor metastasis: Biobehavioral mechanisms**
  Anil K. Sood
  University of Texas
  M.D. Anderson Cancer Center
- **Sleep, circadian, hormonal dysregulation and breast cancer survival**
  David Spiegel
  Stanford University
- **Psychoneuroimmunology and cervical cancer**
  Lari B. Wenzel
  University of California, Irvine