

Stress Influences on Anoikis

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Abstract

It long has been suspected that psychosocial factors affect cancer development and progression. Although the connections between stress and cancer causation are not strong, epidemiologic and clinical studies have provided strong links between cancer progression and several stress-related factors including chronic stress, depression, and social isolation. Recent molecular and biological studies have identified specific signaling pathways that influence cancer growth and metastasis. In particular, stress hormones can have a significant impact on protecting cancer cells from undergoing the anoikis form of programmed cell death, thus, providing a mechanistic advantage for metastasis. This review provides an overview of the relationship between psychosocial factors and the avoidance of anoikis by cancer cells. *Cancer Prev Res*; 4(4); 481-5. ©2011 AACR.

Over 3 decades ago, Engel recognized that biological factors alone cannot account for all changes in physical health and that social, psychological, and behavioral dimensions must be considered in a comprehensive biopsychosocial or "mind-body" model of illness (1). Even earlier, Selye reported that patients with a variety of ailments manifested many similar symptoms, which he referred to as the stress syndrome (2, 3). McEwen refers to the long-term effects of the physiologic response to stress as allostatic load (4). Growing evidence supports the role of psychosocial stress in a wide variety of human ailments including cardiovascular diseases and cancer (5). Whether psychological stress increases the risk for developing cancer, however, remains unclear (6, 7). Some studies have found that severe stress (e.g., death of a spouse or child) may increase cancer risk (8, 9); many studies, however, have not shown a convincing link (8, 10). A recent meta-analysis did not find a significant effect for exposure to stressors on cancer incidence, but it suggested that certain personality and coping styles were associated with increased cancer risk (11). More compelling and consistent is the evidence for the impact of psychosocial factors on clinical outcomes, particularly mortality, following a cancer diagnosis (11, 12).

To fully understand the impact of chronic stress on human health, it is important to dissect the underlying biological mechanisms of this impact. The archetypal components of the stress response involve the sympathetic

nervous system (SNS) and the hypothalamic pituitary adrenal (HPA) axis (12). Stress can be acute (short-lived) or chronic (repetitive or occurring over an extended period of time; ref. 13). Under chronic stress conditions, the body remains in a constant state of "overdrive," with deleterious downstream effects on regulation of stress response systems and on many organ systems. The physiologic stress response is thought to be one of the likely mediators of the effects of psychosocial factors on cancer progression. The overall stress response involves activation of several physiologic systems including the autonomic nervous system (ANS) and the HPA axis. The "fight or flight" response is elicited by the production of mediators, such as norepinephrine and epinephrine, from the SNS and adrenal medulla. The HPA response includes release of corticotropin-releasing hormone from the hypothalamus, which induces secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary, resulting in downstream release of glucocorticoids, such as cortisol, from the adrenal cortex.

Neuroendocrine mediators can modulate cellular function in many of the peripheral tissue sites most relevant to cancer onset and progression. For example, catecholamines from the SNS play physiologically relevant roles in regulating the microenvironment of peripheral organs (peripheral to the central nervous system) such as the ovaries, which illustrate this concept very well as it applies to cancers of the reproductive system. Overall concentrations of catecholamines are substantially higher in the ovary than in plasma (14). Moreover, catecholamine levels in the ovary are known to be increased in response to stress due to increased sympathetic activity, which has been shown to result in the appearance of precystic follicles (15-18). Similarly, catecholamines are present at substantially higher levels in the bone marrow microenvironment and are secreted from both nerve endings and bone marrow cells (19). We and others have demonstrated how activation of stress pathways in preclinical (animal) models and

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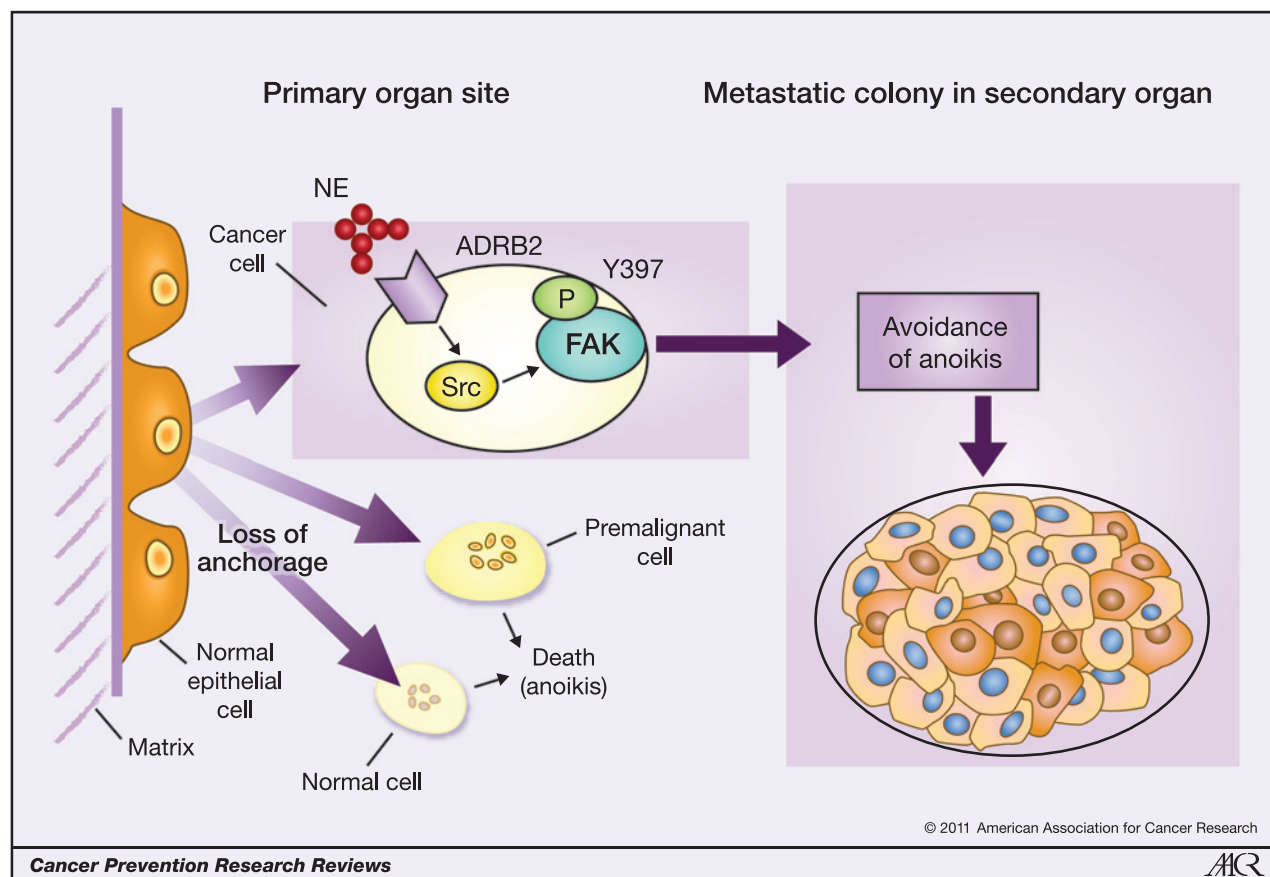


Figure 1. Adrenergic pathway–mediated protection of cancer cells from anoikis. Normal-appearing cells (left) typically are attached to their matrix and each other and undergo programmed cell death via anoikis upon detachment (bottom). Detached premalignant cells have a similar fate (middle). Cancer cells, however, can survive despite loss of attachment (top). As mediated by ADRB2s, exposure of cancer cells to stress hormones such as norepinephrine (NE) leads to activation, or phosphorylation, of FAK^{Y397}, leading in turn to anoikis avoidance and metastasis (right).

human patients results in elevated stress hormones in multiple organs and the tumor microenvironment (20–22). Additional neuroendocrine factors, including dopamine, prolactin, nerve growth factor (NGF), substance P, and oxytocin, are also modulated under chronic stress states (23, 24).

Given emerging clinical evidence for the effects of stress on cancer progression, we and others have examined potential effects of stress mediators on various steps involved in metastasis. It is important to understand such mechanistic influences since the major cause of death from cancer is metastases that are resistant to conventional therapy, as they very frequently are (25). To the extent that stress mediators influence cancer progression, a mechanistic and biological understanding of these effects could identify new opportunities for improving the outcome of cancer patients. Primary neoplasms are biologically heterogeneous, and the process of metastasis consists of a series of sequential and selective steps that few cells can successfully complete. The outcome of cancer metastasis depends on multiple interactions between metastatic cells and homeostatic mechanisms that are unique to a given organ micro-

environment (26). Research over the last 20 years has demonstrated that neuroendocrine stress mediators might enhance cancer pathogenesis by inhibiting antitumor immune responses (27). For example, findings of our group and others have shown that higher levels of social isolation, stress, and/or distress tend to be related to poorer cellular immune function in both the peripheral blood and tumor microenvironment of breast and ovarian cancer patients (28–30).

Given the uncertain role of immune pathways in advanced cancer, we and others have also explored the potential impact of stress hormones on other steps in the metastatic cascade (31). Various stress hormones have been shown to influence the metastatic steps of angiogenesis, invasion, migration, and proliferation (12, 20). SNS activity involving catecholamines can directly enhance the pathogenesis of ovarian carcinoma by upregulating angiogenic pathways (e.g., VEGF and interleukin-6 and -8 levels) in the tumor microenvironment (20, 32–34). These effects were mediated through activation of tumor cell $\beta 2$ adrenergic receptors (ADRB2) and the associated cyclic AMP (cAMP)–protein kinase A (PKA) signaling pathway.

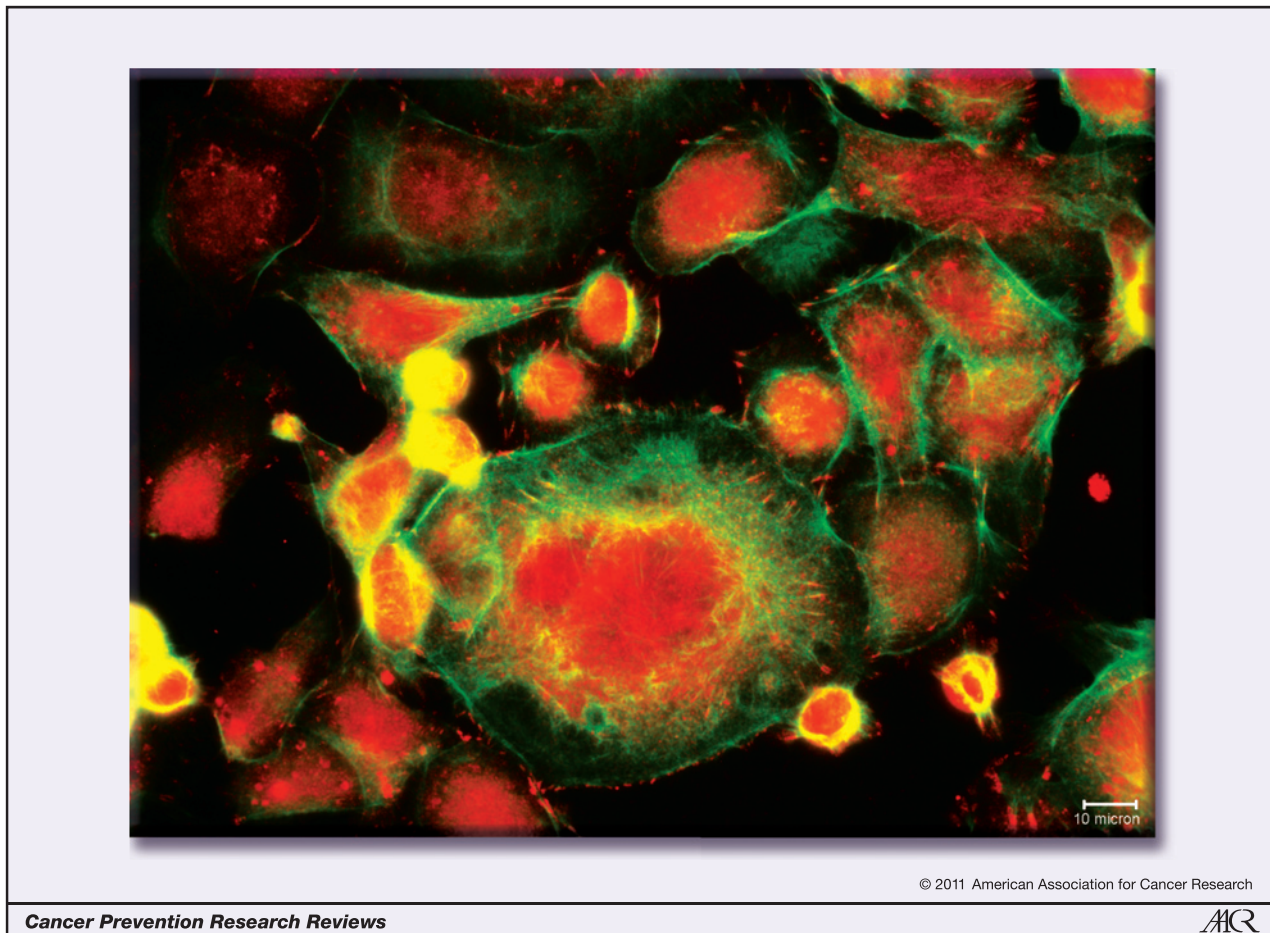


Figure 2. SKOV3ip1 ovarian cancer cells have increased pFAK^{Y397} (red) localized to focal adhesions following treatment with norepinephrine. Immunofluorescence staining identified pFAK^{Y397} and actin (green).

We recently discovered another mechanism by which adrenergic signaling can contribute to metastasis, a mechanism that influences anoikis (Fig. 1). Normal tissues reflect a balance of cellular proliferation, differentiation, and apoptosis. The extracellular matrix plays a critical role in maintaining this balance by inducing survival signaling through integrins or by growth factor signaling. Anoikis is a form of programmed cell death which is induced by anchorage-dependent cells detaching from the surrounding extracellular matrix (35). Resistance to anoikis is a hallmark of malignant transformation, affording tumor cells increased survival times in the absence of matrix attachment and facilitating migration, reattachment, and colonization of secondary sites (Fig. 1; refs. 36, 37). Most of the characteristics reflecting apoptotic cells, such as nuclear fragmentation and membrane blebbing, are also observed during anoikis. Nonadrenergic factors that contribute to protection from anoikis include overexpression of oncogenes, such as *ras*, *raf*, and *src*, and downregulation of tumor suppressor genes such as *PTEN* and *TP53* (encoding p53; ref. 38).

To determine whether the adrenergic hormones (catecholamines) might also inhibit anoikis via focal adhesion

kinase (FAK) activation, we analyzed ovarian cancer cells maintained in poly-HEMA-coated tissue culture plates, which allows for anchorage-independent growth. Exposure to stress concentrations of either epinephrine or norepinephrine resulted in significant inhibition of anoikis (39). On the basis of the known effects of some neuropeptides, such as bombesin, on FAK (40), we considered whether FAK might be involved in the tumor-promoting effects of chronic stress. Following activation by integrins, FAK becomes phosphorylated and associates with several other intracellular signaling molecules. This convergence of signaling by FAK plays an important role in tumor cell survival and may play a significant role in avoidance of anoikis. We and others have previously reported high total and activated FAK levels in ovarian and other cancers (41). Norepinephrine treatment resulted in a rapid and dose-dependent increase in phosphorylated FAK^{Y397} (pFAK^{Y397}), which was localized to focal adhesions (Fig. 2). This increase in pFAK^{Y397} was mediated through ADRB2 since both broad β -blockers (propranolol) and ADRB2-specific blockers (butoxamine) abrogated the norepinephrine-mediated increase in FAK activation. The

β -blockers also blocked the protection that adrenergic stimulation provides cancer cells against anoikis.

To elucidate the underlying signaling pathways responsible for FAK activation, we considered the potential role of Src kinase. Indeed, a number of *in vitro* kinase assays provided direct proof for the role of Src kinase in activating FAK^{Y397} in response to norepinephrine (39). In a biological setting, cancer cells must avoid anoikis during the process of metastasis after detachment from the primary organ site. In the context of ovarian cancer, metastasis frequently occurs by cancer cell dissemination via ascitic fluid present within the peritoneal cavity. In an orthotopic mouse model of ovarian cancer with ascites, stress induced by daily physical restraint significantly reduced the number of apoptotic cells, suggesting a reduction of anoikis. Similar effects were observed with the β -agonist isoproterenol. Both chronic stress and isoproterenol resulted in increased phosphorylation of FAK^{Y397}, which was blocked by propranolol. FAK silencing using small interfering RNA (siRNA) delivered via 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) nanoliposomes also blocked stress-mediated protection against anoikis.

In the clinical setting, we often examine levels of depression as a way to parallel these preclinical stress findings since depression is frequently linked to chronic stress (42, 43) and has been related to elevated stress hormones such as norepinephrine (22, 44). To determine whether stress hormones could be linked to FAK activation in human biology, a series of ovarian cancers were examined. In these studies, high levels of depression (based on Center for Epidemiological Studies Depression scale score >16) were associated with increased pFAK^{Y397} expression. Similarly, high norepinephrine content in the tumor was associated with increased pFAK^{Y397} expression. Therefore, these results were complementary to the preclinical findings described above.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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