

## Short Communication

## Social isolation is associated with elevated tumor norepinephrine in ovarian carcinoma patients

Susan K. Lutgendorf<sup>a,b,c,d,\*</sup>, Koen DeGeest<sup>b</sup>, Laila Dahmouh<sup>e</sup>, Donna Farley<sup>f</sup>, Frank Penedo<sup>g</sup>, David Bender<sup>b</sup>, Michael Goodheart<sup>b</sup>, Thomas E. Buekers<sup>h</sup>, Luis Mendez<sup>i</sup>, Gina Krueger<sup>j</sup>, Lauren Clevenger<sup>a</sup>, David M. Lubaroff<sup>c,d,k</sup>, Anil K. Sood<sup>l</sup>, Steve W. Cole<sup>m</sup>

<sup>a</sup> Department of Psychology, University of Iowa

<sup>b</sup> Department of Obstetrics & Gynecology, University of Iowa, USA

<sup>c</sup> Department of Urology, University of Iowa, USA

<sup>d</sup> Holden Comprehensive Cancer Center, University of Iowa, USA

<sup>e</sup> Department of Pathology, University of Iowa, USA

<sup>f</sup> Institute of Clinical and Translational Science, University of Iowa, USA

<sup>g</sup> Department of Psychology and Sylvester Comprehensive Cancer Center, University of Miami, USA

<sup>h</sup> Division of Gynecologic Oncology, Henry Ford Hospital, USA

<sup>i</sup> Division of Gynecologic Oncology, Florida International University College of Medicine, USA

<sup>j</sup> Department of Epidemiology, College of Public Health, University of Iowa, USA

<sup>k</sup> Department of Microbiology, University of Iowa, USA

<sup>l</sup> Departments of Gynecologic Oncology and Cancer Biology, MD Anderson Cancer Center, USA

<sup>m</sup> Division of Hematology-Oncology, UCLA School of Medicine, the Norman Cousins Center, Jonsson Comprehensive Cancer Center, and the UCLA Molecular Biology Institute, University of California at Los Angeles, USA

## ARTICLE INFO

## Article history:

Received 12 July 2010

Received in revised form 6 October 2010

Accepted 11 October 2010

Available online 16 October 2010

## Keywords:

Social isolation

Ovarian cancer

Norepinephrine

Beta-adrenergic signaling

Catecholamines

## ABSTRACT

Noradrenergic pathways have been implicated in growth and progression of ovarian cancer. Intratumoral norepinephrine (NE) has been shown to increase with stress in an animal cancer model, but little is known regarding how tumor NE varies with disease stage and with biobehavioral factors in ovarian cancer patients. This study examined relationships between pre-surgical measures of social support, depressed mood, perceived stress, anxiety, tumor histology and tumor catecholamine (NE and epinephrine [E]) levels among 68 ovarian cancer patients. We also examined whether associations observed between biobehavioral measures and tumor catecholamines extended to other compartments. Higher NE levels were found in advanced stage ( $p = 0.006$ ) and higher grade ( $p = 0.001$ ) tumors. Adjusting for stage, grade, and peri-surgical beta blockers, patients with a perceived lack of social support had significantly higher tumor NE ( $\beta = -0.29$ ,  $p = 0.012$ ). A similar trend was seen for social support and ascites NE (adjusting for stage, peri-surgical beta blockers and caffeine:  $\beta = -0.50$ ,  $p = 0.075$ ), but not for plasma NE. Other biobehavioral factors were not related to tumor, ascites, or plasma NE ( $p$  values  $>0.21$ ). Tumor E was undetectable in the majority of tumors and thus E was not further analyzed. In summary, these results suggest that tumor NE provides distinct information from circulating plasma concentrations. Tumor NE levels were elevated in relationship to tumor grade and stage. Low subjective social support was associated with elevated intratumoral NE. As beta-adrenergic signaling is related to key biological pathways involved in tumor growth, these findings may have implications for patient outcomes in ovarian cancer.

© 2010 Elsevier Inc. All rights reserved.

## 1. Introduction

Clinical and epidemiologic studies have demonstrated positive associations between stress and cancer progression (Chida et al., 2008; Kroenke et al., 2006; Sprehn et al., 2009) although findings

are inconsistent. A key component of the stress response involves activation of the sympathetic nervous system (SNS) and production of mediators such as the catecholamines norepinephrine (NE) and epinephrine (E), which arise both from the SNS and the adrenal medulla (McEwen, 2007). Animal-based research has shown that stress can increase levels of intratumoral NE (Shahzad et al., 2010) as well as NE in the ovary and organs that are typical metastatic sites for ovarian cancer such as spleen and omentum (Thaker et al., 2006). Beta-adrenergic signaling has been shown

\* Corresponding author at: University of Iowa, Department of Psychology, E11 Seashore Hall, Iowa City, IA 52242, USA. Fax: +1 319 335 0191.

E-mail address: [susan-lutgendorf@uiowa.edu](mailto:susan-lutgendorf@uiowa.edu) (S.K. Lutgendorf).

to enhance biological processes involved in cancer progression such as angiogenesis, invasion, and metastasis (Chakraborty et al., 2009; Sood et al., 2006, 2010; Thaker et al., 2006; Sood et al., 2010; Shahzad et al., 2010). NE and E have been shown to stimulate the production of pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6), and pro-invasive molecules such as matrix metalloproteinases 2 and 9 (MMP2, MMP-9) from ovarian and other cancer cells, thus increasing the invasive and metastatic potential of these cells (Lutgendorf et al., 2003, 2008; Nilsson et al., 2007; Sood et al., 2006; Thaker et al., 2006; Yang et al., 2002, 2006). Beta-adrenergic signaling also promotes ovarian cancer cell survival by inhibiting anoikis, the normal process of apoptosis that occurs when cells are separated from the extracellular matrix (ECM) (Sood et al., 2010).

High levels of social isolation have been consistently associated with increased risk for morbidity and mortality, with statistical effect sizes comparable to those of standard health risk factors such as smoking, blood pressure, and obesity (House et al., 1988). Elevated catecholamine levels have been observed in individuals with low social support, chronic stress, and depression (Esler et al., 1982; Hamer et al., 2007; Hughes et al., 2004; Seeman and McEwen, 1996; Weiner, 1992). In patients with ovarian cancer, poor social support has also been linked to higher levels of angiogenic cytokines including VEGF and IL-6, both in peripheral blood and in the tumor micro-environment (Costanzo et al., 2005; Lutgendorf et al., 2002, 2008). We previously reported in a small sample of 10 ovarian cancer patients that those with poor social support and high depression had significantly higher levels of tumor (but not plasma) NE as compared to patients with high social support and low depression (Lutgendorf et al., 2009). These catecholamine patterns paralleled alterations in activity in tumor tissue of beta-adrenergically linked transcription control pathways mediating processes such as inflammation, metastatic capacity, and cell proliferation (Lutgendorf et al., 2009). Other than this small previous study, the relationship of psychosocial factors and tumor catecholamines has not been examined.

To address this knowledge gap, we examined associations between social support, depression, perceived stress and levels of NE and E in primary ovarian tumor tissue. We also examined the relationship between catecholamines and tumor stage and grade, which has not been previously characterized. Based on our previous work (Lutgendorf et al., 2009), we hypothesized that lower social support, greater depression and greater perceived stress would be associated with higher catecholamine levels in tumor tissue, but not in circulating blood.

## 2. Methods

### 2.1. Participants

Women over 18 years of age with a new diagnosis of a pelvic or abdominal mass suspected to be ovarian cancer were recruited. Inclusion was confirmed by histologic diagnosis of primary invasive epithelial ovarian, peritoneal, or fallopian tube cancer. Patients were excluded for primary cancer of another organ site, a non-epithelial ovarian tumor, an ovarian tumor of low malignant potential, use of systemic corticosteroid medication in the last 4 months, chronic use of beta-blockers, or comorbidities known to alter the immune response. Eighteen patients had been placed on beta-blockers pre-operatively (usually 1–12 days before their surgery). Although there was no significant difference in tumor NE levels for patients taking vs. not taking peri-surgical beta-blockers (Mann-Whitney  $Z = 1.27$ ,  $p = 0.204$ ), this was used as a covariate in statistical models as a conservative measure. Mucinous tumors ( $n = 3$ ) were eliminated because of difficulty in accurate catecholamine analysis. Sixty-eight ovarian cancer patients with tumor

samples, verified as positive for ovarian carcinoma by the study pathologist, were included. Plasma catecholamines were available for 53 of these patients, and 22 had ascites (accumulated fluid in the peritoneal cavity). This research was approved by the Institutional Review Boards at each site. Psychosocial questionnaires were completed between the pre-operative appointment and surgery. Blood sampling was done in the pre-surgical waiting area approximately 2 h before surgery, between approximately 6:00 and 11:30 AM; patients had routinely abstained from intake of anything by mouth since midnight.

### 2.2. Biobehavioral, demographic, and medical assessments

#### 2.2.1. Depressed mood

The Center for Epidemiological Studies Depression Scale (CES-D) is a 20-item measure that assesses depressive symptomatology over the last week (Radloff, 1977). Scores of 16 or higher have been associated with clinical depression (Ensel, 1986; Radloff, 1977).

#### 2.2.2. Social support/isolation

The Social Provisions Scale (SPS) is a 24-item self-report scale measuring the extent to which social relationships are perceived as supportive (Russell et al., 1984).

#### 2.2.3. Distress

The Incredibly Short POMS includes 6 adjectives reflecting the Profile of Mood States subscales (anxiety, depression, anger, vigor, fatigue, confusion) and rated on a 5-point scale from 0 (not at all) to 4 (extremely) according to current mood (Dean et al., 1989; Sacham, 1983). This was administered at the time of blood draw to provide a rapid assessment of pre-surgical mood. The anxiety question was used in this study to rule out the potential confound of pre-surgical anxiety.

#### 2.2.4. Perceived stress

The Perceived Stress Scale (PSS) is a 14-item measure designed to assess the degree to which situations in one's life over the past month are perceived as stressful (Cohen et al., 1983). The instrument provides a global measure of stress, examining the degree to which one's life is perceived as unpredictable, uncontrollable, and beyond one's capacity to cope. This measure has been associated with vulnerability to development of infectious disease in healthy individuals (Cohen et al., 1983).

#### 2.2.5. Demographic, health behavior, and clinical information

Clinical and histopathologic information was obtained from medical records. Information on demographic characteristics and health behaviors such as hours of sleep, smoking, alcohol and caffeine intake during the 7 days before surgery were provided by patient health report.

### 2.3. Catecholamines

Catecholamine levels were determined by high performance liquid chromatography with electrochemical detection (HPLC-ED) as previously described (Hoffman et al., 2002; Lutgendorf et al., 2009). Briefly, plasma catecholamines were collected in chilled EDTA Vacutainer tubes (BD Biosciences, Franklin Lakes, NJ), kept on ice prior to centrifugation, and then stored at  $-80^{\circ}\text{C}$ . Plasma catecholamines were adsorbed onto activated alumina at pH 8.6, washed, and eluted with dilute acid prior to injection onto a reverse-phase column to separate the individual catecholamines. Ascites was centrifuged at 20,200g for 5 min to remove particulate matter and catecholamines present in 1 mL of clear fluid were adsorbed onto activated alumina and treated as above. Electrochemical detection was done using a Coulochem II Dual Potentiostat

Electrochemical Detector (ESA, Chelmsford, MA). A calibration curve using “blank” human plasma or ascites samples (dialyzed to remove endogenous catecholamines) and linear regression analysis were used to determine catecholamine concentrations in the samples. Interassay and intra-assay coefficients of variation were 3.4% and 3.1%, respectively. The lower limit of detection was 40 pg/mL for NE and 20 pg/mL for E.

Dissected 2–3 mm<sup>3</sup> tumor tissue samples were snap frozen in liquid nitrogen and kept in liquid nitrogen until assay. For analysis, samples were pulverized using liquid nitrogen, and maintained on dry ice. Tissue powder was homogenized, neutralized, and adsorbed onto acid washed alumina as previously described (Lutgendorf et al., 2009). The alumina was washed twice with water and catecholamines were eluted with 1 ml 0.05 M perchloric acid containing 0.1 mM sodium metabisulfite. After microfiltration, eluate was diluted in 4% acetic acid, and catecholamines were resolved using an Aquasil C18 4 μm (100 × 4.6 mm) HPLC column (Thermo Electron Corp., Bellefonte, PA), followed by electrochemical detection as described above. Tumor E was not detectable in 88.1% of patients and tumor NE was not detectable in 33.8% of patients. Catecholamine levels for these patients were set at the lowest detectable level of the assay which was 0.1 pg/mg for NE and 0.2 pg/mg for E.

#### 2.4. Statistical analyses

SPSS version 17.0 (Statistical Program for the Social Sciences, Chicago, IL) was used for data analysis. Catecholamine concentrations in each compartment were tested for simple association with continuous variables using Pearson correlations and for association with categorical variables using Chi Squared tests or analyses of variance (ANOVA). General linear models were used to examine relationships of catecholamine concentration according to stage and grade (adjusting for use of peri-surgical beta blockade). For hypotheses involving biobehavioral data, hierarchical linear regressions, adjusting for stage, grade, peri-surgical beta blockade, and where relevant, caffeine intake, were used to test hypotheses. Analyses for tumor E were performed with non-parametric statistics due to a positively skewed distribution. All other biological variables were log transformed to normalize distributions. Because there was one outlier in the social support data, significant findings for this variable were also analyzed by rank order in a secondary analysis. Rank-based analyses mitigate the effect of outliers (Miller, 1998). A *p* value of <0.05 was considered statistically significant.

### 3. Results

#### 3.1. Participant characteristics

Table 1 presents demographic characteristics and mean catecholamine levels by compartment. There were no significant associations between age or health behaviors and NE in tumor tissue or peripheral blood (all *p* values >0.12). Caffeine consumption was associated with higher levels of ascites NE (*p* = .059), and therefore was treated as a covariate in analyses involving ascites NE. Pre-surgical anxiety was examined as a potential confound, but was not significantly related to catecholamines in any compartment (all *p* values >0.28). There were no significant associations of relationship status (single vs. divorced/separated/widowed vs. married/living with partner) with catecholamines in any compartment (*p* > 0.34).

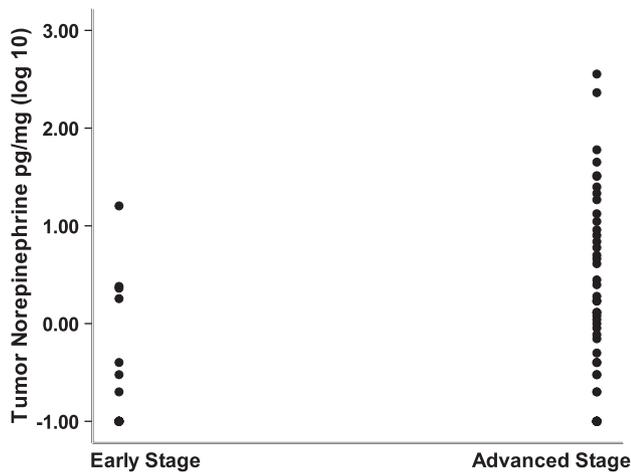
#### 3.2. Catecholamines and tumor histology

Intratumoral NE levels differed substantially as a function of tumor stage, with Stage I and II tumors showing comparatively

**Table 1**  
Patient characteristics.

Measure	Cancer patients (N = 68)
<i>Age in years</i>	
Mean (standard deviation)	59.4 (12.3)
<i>Income</i>	
<10,000	% of patients 9.4
10,000–20,000	15.1
20,001–40,000	34.0
40,001–60,000	11.3
60,001–80,000	20.8
>80,000	9.4
<i>Marital status</i>	
Single	10.9
Divorced/separated	15.7
Widowed	4.7
Married/living with partner	68.7
<i>Race</i>	
American Indian/Alaskan Native	1.5
African American	1.5
Caucasian	97.0
<i>Ethnicity</i>	
Hispanic	10.3
Non-Hispanic	89.7
<i>Stage</i>	
I	17.6
II	7.4
III	66.2
IV	8.8
<i>Grade</i>	
1	11.8
2	10.3
3	77.9
<i>Tumor histology</i>	
Serous	82.4
Endometrioid	13.2
Clear cell	1.5
Other	3.0
<i>Cytoreduction</i>	
Optimal	77.9
Suboptimal	22.1
<i>mean (SD)</i>	
Plasma NE	573.32 pg/mL (294.80)
Plasma E	83.17 pg/mL (67.64)
Ascites NE	532.81 pg/mL (263.96)
Ascites E	50.33 pg/mL (34.74)
Tumor NE	13.79 pg/mg (51.56)
Tumor E	.10 pg/mg (.39)

low concentrations (Stage I = 1.6 ± 4.56 pg/mg, Stage II = 1.04 ± .120 pg/mg) and Stage III and IV tumors showing substantially greater concentrations (Stage III = 18.29 ± 62.65 pg/mg, Stage IV = 14.26 ± 17.87 pg/mg). The overall *F* value approached significance *F*(3,64) = 2.65, *p* = 0.056, and the contrast between advanced-stage (III and IV) and early-stage (I and II) tumors was highly significant, *F*(1,65) = 8.09, *p* = 0.006. (Fig. 1). Tumors of high grade also had significantly higher NE than low grade tumors, *F*(1,65) = 12.72, *p* = 0.001. These analyses adjusted for peri-surgical beta blockade. E levels fell below the assay limit of detection in the majority of tumor samples. However, among the minority of tumor samples in which it could be measured (11.9%), there was a possible trend towards elevated E in advanced stage tumors (Mann–Whitney *z* = −1.74, *p* = 0.08). Tumor E was not related to grade (*p* = 0.11). As tumor E was undetectable in the majority of patients, further analyses were not conducted on E in other compartments. Plasma NE did not vary according to disease stage (*p* = 0.35) or grade (*p* = 0.76). Plasma and tumor NE levels were not related, *r* = 0.08, *p* = 0.55. Ascites



**Fig. 1.** Tumor NE in early and advanced stage ovarian cancer patients ( $p = 0.006$ ). Ovarian cancer stage is shown on the X-axis and levels of tumor NE are shown on the Y-axis.

NE was elevated in high grade compared to grade 1 and 2 tumors ( $281.84 \pm 56.99$  pg/mL vs  $524.81 \pm 98.85$  pg/mL,  $p = 0.032$ ) and in advanced-stage ( $560.66 \pm 254.11$  pg/mL) compared to early-stage patients ( $167.00$  pg/mL). Due to the relative absence of ascites in patients with early-stage disease ( $n = 1$ ), data for stage were not analyzed statistically. Ascites NE was positively associated with both plasma ( $r = 0.36$ ,  $p = .13$ ,  $n = 19$ ) and tumor NE ( $r = 0.35$ ,  $p = .11$ ,  $n = 22$ ) but these associations were not significant.

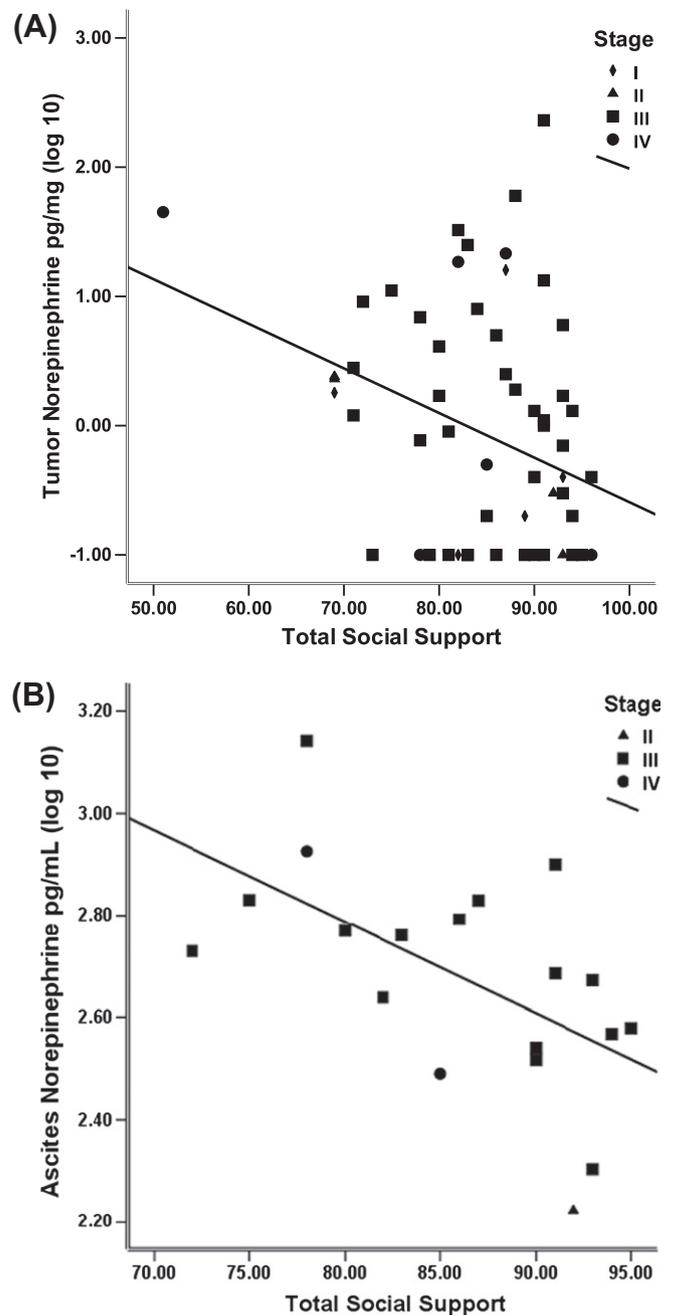
### 3.3. Biobehavioral factors and catecholamines in three compartments

As seen in Fig. 2A, patients with higher levels of social support had lower tumor NE, adjusting for stage, grade, and peri-surgical beta blockade ( $\beta = -0.29$ ,  $p = 0.012$ ). Similar results emerged from rank-based analyses mitigating the influence of the one social support outlier, adjusting for the same covariates,  $\beta = -0.30$ ,  $p = 0.009$ . Neither perceived stress nor depressed mood was significantly associated with tumor NE (all  $p$  values  $>0.21$ ). Furthermore, plasma NE was not related to any biobehavioral variable (all  $p$  values  $>0.31$ ).

We then examined whether relationships between social support and tumor catecholamine activity would also be observed in the ascites fluid surrounding the tumor. Social support showed an even greater magnitude of association with ascites NE than seen with tumor NE, adjusting for stage, caffeine intake, and use of peri-surgical beta blockers. However, this relationship did not reach statistical significance ( $\beta = -0.50$ ,  $p = 0.075$ ,  $n = 19$ ), potentially due to the smaller sample size of patients with ascites. (Fig. 2B). Because of small sample size, grade was included in a parallel model, with similar results ( $\beta = -0.49$ ,  $p = 0.06$ ,  $n = 19$ ).

## 4. Discussion

The key findings of this study are that intratumoral NE levels in primary ovarian carcinomas are linked to both disease severity and patient psychosocial characteristics. Tumor NE was elevated in patients with advanced stage disease and higher grade pathology. Independent of grade or stage, tumor NE levels were also higher in patients reporting lower perceived social support at the time of surgical resection. Similar patterns were seen for ascites NE although relationships did not reach statistical significance, potentially due to the limited numbers of patients with ascites. Social support was not related to plasma catecholamines. Depression,



**Fig. 2.** Tumor NE (A) and ascites NE (B) as a function of perceived social support. Norepinephrine values are transformed to  $\log_{10}$ . Adjusting for stage, grade, and peri-surgical beta blockers, patients with poor social support had significantly higher tumor NE ( $\beta = -0.29$ ,  $p = 0.012$ ). A similar trend was seen for social support and ascites NE (adjusting for stage, peri-surgical beta blockers and caffeine:  $\beta = -0.50$ ,  $p = 0.075$ ,  $n = 19$ ). Higher scores on the X-axis represent greater social support.

generalized perceived stress, pre-surgical anxiety, and use of peri-surgical beta blockers were not associated with tumor NE. These findings extend our previous report of elevated tumor catecholamines accompanying biobehavioral risk factors in a small sample of ovarian cancer patients (Lutgendorf et al., 2009). They also extend pre-clinical experiments demonstrating that behavioral stress increases intratumoral NE in an orthotopic mouse model of ovarian cancer (Shahzad et al., 2010), and also increases NE in organs that are typical metastatic sites for ovarian cancer (Thaker et al., 2006). To the extent that the intratumoral catecholamine differences observed here affect biological pathways involved in

tumor progression such as angiogenesis, invasion, anoikis, transcription pathway activation, etc. (Chakroborty et al., 2009; Lutgendorf et al., 2009; Sood et al., 2010; Sloan et al., 2010), the present results potentially shed light on relationships between psychosocial distress and increased risk of metastasis (Thaker et al., 2006).

Although this study demonstrates that biobehavioral factors are related to tumor NE levels, the biological mechanisms underlying these effects will require further definition. There are direct connections from the central nervous system to the ovary via the sympathetic nervous system (SNS), as well as neural feedback from the ovary to the hypothalamus (Aguado, 2002). These connections influence the estrus cycle (Aguado, 2002; Ben-Jonathan et al., 1984), but also may provide a neural pathway by which biobehavioral states could alter ovarian biology. Stress has been shown to upregulate the arborization of sympathetic neural fibers (e.g., in lymphoid organs) (Sloan et al., 2008), and stress-induced enhancement of tumor vascularization (Thaker et al., 2006) may also increase innervation via increased density of peri-vascular nerve fibers. In addition to conveying biobehavioral influences into the healthy ovary and ovarian cancer tissues, these dynamics could also establish a positive feedback loop for sympathetic activity and tumor growth. The basis for increased NE concentrations in ascites also remains to be determined, although increased spill-over from peri-vascular neuro-muscular junctions represents a plausible biological mechanism (Weiner, 1992).

In pre-clinical models, catecholamines have been shown to enhance the expression of genes involved in angiogenesis (e.g., *VEGF*, *IL6*) and tissue invasion (*MMP2*, *MMP9*) in ovarian and nasopharyngeal carcinoma cells (Nilsson et al., 2007; Sood et al., 2006; Yang et al., 2006) and to directly activate angiogenesis promoting molecules such as signal transducer and activator of transcription factor-3 (STAT-3) which has downstream effects on cell proliferation, survival, and angiogenesis, as well as inhibition of apoptosis (Landen et al., 2007). Catecholamines were also observed to protect tumor cells from anoikis by activating focal adhesion kinase (FAK), a protein tyrosine kinase that mediates physical attachment of cells to their ECM (Sood et al., 2010). In animal models, catecholamines increase angiogenesis, invasion, tumor weight and number of nodules (Thaker et al., 2006), and protect tumor cells from anoikis (Sood et al., 2010). Beta adrenergic activation of the cAMP-PKA signaling pathway via the beta-2 adrenergic receptor appears to be the major pathway mediating these effects (Sood et al., 2010; Thaker et al., 2006). Additional signaling pathways are likely also affected by catecholamines but have not been fully characterized.

What accounts for the relationship of social isolation with tumor NE? Socially isolated individuals are thought to have less availability of stress-reducing interpersonal resources such as emotional or instrumental support which would normally serve to bolster active coping efforts and thereby reduce stress (Waite and Hughes, 1999). Although effects on health have been thought to derive partially from the fact that social isolation often leads to stress or depression (Cacioppo et al., 2006), in our data it was social isolation and not stress or depression which was related to tumor catecholamines. The ability to elicit social support may be a stable individual difference characteristic (Smith and MacKenzie, 2006) and perceived social isolation may thus reflect an ongoing state of stress vulnerability with concomitant SNS activation that is not necessarily represented in more localized measures of stressful life experience. Such an ongoing vulnerability may result in greater integrated accumulation of catecholamines over time.

It is notable that stronger relationships were seen between social support and tumor NE rather than plasma NE. This finding, along with the fact that there was not a relationship between levels of NE in plasma and tumor implies that catecholamines in peripheral circulation are likely not responsible for the bulk of the intratumoral catecholamine levels. These findings also suggest that

direct neural linkages between the CNS and ovary may operate differently than the SNS dynamics giving rise to the plasma catecholamine signal. It is also possible that catecholamine accumulation may differ in these two compartments, as the hypoxic and acidic tumor environment may enhance preservation of catecholamines (Miki and Sudo, 1998). Our data suggest that measurement of stress factors in cancer research should be performed in the compartment of interest and that extrapolation of circulating catecholamine measurements to the tumor micro-environment would be misleading.

#### 4.1. Limitations

Blood sampling was done in a private pre-surgical examination room most often after the patient had been sitting for at least 5 min waiting for a nurse. However, because this was a clinical setting, movement artifacts may have been introduced for some patients. Tumors were harvested for catecholamine levels approximately 2–3 h later during surgery, potentially affecting direct comparisons between plasma and tumor catecholamine levels. Although use of peri-surgical beta blockers was not significantly related to levels of tumor NE, residual effects of this pharmacological intervention on results cannot be totally ruled out. No definitive causal conclusions can be drawn from this observational study.

#### 4.2. Conclusions

Social isolation was associated with higher tumor NE among ovarian cancer patients. As beta-adrenergic signaling is related to key pathways involved in tumor growth, these findings may have implications for patient outcomes in ovarian cancer.

#### Conflict of interest statement

The authors have no conflict of interest.

#### Acknowledgments

Grant support: This research was funded in part by grants CA88293, CA104825 and CA104825 Suppl1, CA140933 (S.K.L.), CA109298, CA110793 and the U.T. MD Anderson Cancer Center Ovarian Cancer SPORE (#P50CA083639) (A.K.S.), CA116778 (S.C.).

#### References

- Aguado, L.I., 2002. Role of the central and peripheral nervous system in the ovarian function. *Microsc. Res. Tech.* 59, 462–473.
- Ben-Jonathan, N., Arbogast, L.A., Rhoades, T.A., Bahr, J.M., 1984. Norepinephrine in the rat ovary: ontogeny and de novo synthesis. *Endocrinology* 115, 1426–1431.
- Cacioppo, J.T., Hughes, M.E., Waite, L.J., Hawkley, L.C., Thisted, R.A., 2006. Loneliness as a specific risk factor for depressive symptoms: cross-sectional and longitudinal analyses. *Psychol. Aging* 21 (140–51).
- Chakroborty, D., Sarkar, C., Basu, B., Dasgupta, P.S., Basu, S., 2009. Catecholamines regulate tumor angiogenesis. *Cancer Res.* 69, 3727–3730.
- Chida, Y., Hamer, M., Wardle, J., Steptoe, A., 2008. Do stress-related psychosocial factors contribute to cancer incidence and survival? *Nat. Clin. Pract. Oncol.* 5, 466–475.
- Cohen, S., Kamarck, T., Mermelstein, R., 1983. Global measure of perceived stress. *J. Health Hum. Behav.* 24, 385–396.
- Costanzo, E.S., Lutgendorf, S.K., Sood, A.K., Anderson, B., Sorosky, J., Lubaroff, D.M., 2005. Psychosocial factors and interleukin-6 among women with advanced ovarian cancer. *Cancer* 104, 305–313.
- Dean, J., Whelan, J., Meyers, A., 1989. An incredibly quick way to assess mood states: the incredibly short POMS. In: Paper presented at the Advancement of Applied Sport Psychology, San Antonio, Texas.
- Ensel, W.M. (Ed.), 1986. *Measuring Depression: The CES-D Scale*. Academic Press, New York.
- Eslser, M., Turbott, J., Schwarz, R., Leonard, P., Bobik, A., Skews, H., Jackman, G., 1982. The peripheral kinetics of norepinephrine in depressive illness. *Arch. Gen. Psychiatry* 39, 295–300.

- Hamer, M., Tanaka, G., Okamura, H., Tsuda, A., Steptoe, A., 2007. The effects of depressive symptoms on cardiovascular and catecholamine responses to the induction of depressive mood. *Biol. Psychol.* 74, 20–25.
- Hoffman, R.P., Sinkey, C.A., Dopp, J.M., Phillips, B.G., 2002. Systemic and local adrenergic regulation of muscle glucose utilization during hypoglycemia in healthy subjects. *Diabetes* 51, 734–742.
- House, J.S., Landis, K.R., Umberson, D., 1988. Social relationships and health. *Science* 241, 540–545.
- Hughes, J.W., Watkins, L., Blumenthal, J.A., Kuhn, C., Sherwood, A., 2004. Depression and anxiety symptoms are related to increased 24-hour urinary norepinephrine excretion among healthy middle-aged women. *J. Psychosom. Res.* 57, 353–358.
- Kroenke, C.H., Kubzansky, L.D., Schernhammer, E.S., Holmes, M.D., Kawachi, I., 2006. Social networks, social support, and survival after breast cancer diagnosis. *J. Clin. Oncol.* 24, 1105–1111.
- Landen, C.N., Lin, Y.G., Armaiz-Pena, G.N., Das, P.D., Arevalo, J.M.G., Kamat, A.A., Han, L.Y., Jennings, N.B., Spannuth, W., Thaker, P.K., Lutgendorf, S.K., Savary, C.S., Sanguino, A.M., Lopez-Berestein, G., Cole, S.W., Sood, A.K., 2007. Neuroendocrine modulation of signal transducer and activator of transcription-3 in ovarian cancer. *Cancer Res.* 67, 10389–10396.
- Lutgendorf, S.K., Cole, S., Costanzo, E., Bradley, S., Coffin, J., Jabbari, S., Rainwater, K., Ritchie, J.M., Yang, M., Sood, A.K., 2003. Stress-related mediators stimulate vascular endothelial growth factor secretion by two ovarian cancer cell lines. *Clin. Cancer Res.* 9, 4514–4521.
- Lutgendorf, S.K., DeGeest, K., Sung, C.Y., Arevalo, J.M.G., Penedo, F., Lucci, J.A., Goodheart, M., Lubaroff, D., Farley, D.M., Sood, A.K., Cole, S.W., 2009. Depression, social support, and beta-adrenergic transcription control in human ovarian cancer. *Brain Behav. Immun.* 23, 176–183.
- Lutgendorf, S.K., Johnsen, E.L., Cooper, B., Anderson, B., Sorosky, J.L., Buller, R.E., Sood, A.K., 2002. Vascular endothelial growth factor and social support in patients with ovarian carcinoma. *Cancer* 95, 808–815.
- Lutgendorf, S.K., Lamkin, D.M., Jennings, N.B., Arevalo, J.M.G., Penedo, F., DeGeest, K., Langley, R.R., Lucci III, J.A., Cole, S.W., Lubaroff, D.M., Sood, A.K., 2008. Biobehavioral influences on matrix metalloproteinase expression in ovarian carcinoma. *Clin. Cancer Res.* 14, 6839–6846.
- McEwen, B.S., 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* 87, 873–904.
- Miki, K., Sudo, A., 1998. Effect of urine pH, storage time, and temperature on stability of catecholamines, cortisol and creatinine. *Clin. Chem.* 44, 1759–1762.
- Miller, R., 1998. *Beyond ANOVA*. Chapman & Hall CRC, New York.
- Nilsson, M.B., Armaiz-Pena, G., Takahashi, R., Lin, Y.G., Trevino, J., Li, Y., Jennings, N., Arevalo, J., Lutgendorf, S.K., Gallick, G.E., Sanguino, A.M., Lopez-Berestein, G., Cole, S.W., Sood, A.K., 2007. Stress hormones regulate IL-6 expression by human ovarian carcinoma cells through a SRC-dependent mechanism. *J. Biol. Chem.* 282, 29919–29926.
- Radloff, L., 1977. The CES-D scale: a self-report depression scale for research in the general population. *Appl. Psychol. Meas.* 1, 385–401.
- Russell, D., Cutrona, C., Rose, J., Yurko, K., 1984. Social and emotional loneliness: an examination of Weiss's Typology of Loneliness. *J. Pers. Soc. Psychol.* 6, 1313–1321.
- Sacham, S., 1983. A shortened version of the Profile of Mood States. *J. Pers. Assess.* 47, 305–306.
- Seeman, T., McEwen, B., 1996. Impact of social environment characteristics on neuroendocrine regulation. *Psychosom. Med.* 58, 459–471.
- Shahzad, M.M.K., Arevalo, J.M., Armaiz-Pena, G.N., Lu, C., Stone, R.L., Moreno-Smith, M., Nishimura, M., Lee, J.-W., Jennings, N.B., Bottsford-Miller, J., Vivas-Mejia, P., Lutgendorf, S.K., Lopez-Berestein, G., Bar-Eli, M., Cole, S.W., Sood, A.K., 2010. Stress effects on FOSB and interleukin-8 (IL8) driven ovarian cancer growth and metastasis. *J. Biol. Chem.* (epub ahead of print).
- Sloan, E.K., Capitanio, J.P., Cole, S.W., 2008. Stress-induced remodeling of lymphoid innervation. *Brain Behav. Immun.* 22, 15–21.
- Sloan, E.K., Priceman, S.J., Cox, B.F., Yu, S., Pimentel, M.A., Tangkanangnukul, V., Areval, J.M.G., Morizono, K., Karanikolas, B.D.W., Wu, L., Sood, A.K., Cole, S.W., 2010. The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer Res.* 70, 7042–7052.
- Smith, T.W., MacKenzie, J., 2006. Personality and risk of physical illness. *Annu. Rev. Clin. Psychol.* 2, 435–467.
- Sood, A.K., Armaiz-Pena, G., Halder, J., Nick, A., Stone, R., Hu, W., Carroll, A., Spannuth, W., Deavers, M.T., Allen, J., Han, L., Kamat, A., Shahzad, M., McIntyre, B., Diaz-Montero, C., Jennings, N., Lin, Y., Merritt, W., DeGeest, K., Vivas-Mejia, P., Lopez-Berestein, G., Schaller, M., Cole, S., Lutgendorf, S.K., 2010. Adrenergic modulation of focal adhesion kinase protects human ovarian cancer cells from anoikis. *J. Clin. Invest.* 120, 1515–1523.
- Sood, A.K., Bhaty, R., Kamat, A.A., Landen, C.N., Han, L., Thaker, P.H., Li, Y., Gershenson, D.M., Lutgendorf, S., Cole, S.W., 2006. Stress hormone mediated invasion of ovarian cancer cells. *Clin. Cancer Res.* 12, 369–375.
- Sprehn, G.C., Chambers, J.E., Saykin, A.J., Konski, A., Johnstone, P.A., 2009. Decreased cancer survival in individuals separated at time of diagnosis: critical period for cancer pathophysiology? *Cancer* 115, 5108–5116.
- Thaker, P., Han, L.Y., Kamat, A.A., Arevalo, J.M., Takahashi, R., Lu, C., Jennings, N.B., Armaiz-Pena, G., Bankson, J.A., Ravoori, M., Merritt, W.M., Lin, Y.G., Mangala, L.S., Kim, T.J., Coleman, R.L., Landen, C.N., Li, Y., Felix, E., Sanguino, A.M., Newman, R.A., Lloyd, M., Gershenson, D.M., Kundra, V., Lopez-Berestein, G., Lutgendorf, S.K., Cole, S.W., Sood, A.K., 2006. Chronic stress promotes tumor growth and metastasis in ovarian carcinoma. *Nat. Med.* 12, 939–944.
- Waite, L.J., Hughes, M.E., 1999. At risk on the cusp of old age: living arrangements and functional status among black, white and Hispanic adults. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 54, S136–S144.
- Weiner, H., 1992. *Perturbing the Organism: The Biology of Stressful Experience*. University of Chicago Press, Chicago.
- Yang, E., Bane, C.M., MacCallum, R.C., Kiecolt-Glaser, J.K., Malarkey, W.B., Glaser, R., 2002. Stress-related modulation of matrix metalloproteinase expression. *J. Neuroimmunol.* 133, 144–150.
- Yang, E.V., Sood, A.K., Chen, M., Li, Y., Eubank, T.D., Marsh, C.B., Jewell, S., Flavahan, N.A., Morrison, C., Yeh, P.E., Lemeshow, S., Glaser, R., 2006. Norepinephrine up-regulates the expression of vascular endothelial growth factor, matrix metalloproteinase-2, and MMP-9 in nasopharyngeal carcinoma tumor cells. *Cancer Res.* 66, 10357–10364.