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Social Isolation is associated with Elevated Tumor Norepinephrine in Ovarian Carcinoma Patients

Susan K. Lutgendorf^{1,2,3,4,*}, Koen DeGeest², Laila Dahmouch⁵, Donna Farley⁶, Frank Penedo⁷, David Bender², Michael Goodheart², Thomas E. Buekers⁸, Luis Mendez⁹, Gina Krueger¹⁰, Lauren Clevenger¹, David M. Lubaroff^{3,4,11}, Anil K. Sood¹², and Steve W. Cole¹³

¹Department of Psychology, University of Iowa

²Department of Obstetrics & Gynecology, University of Iowa

³Department of Urology, University of Iowa

⁴Holden Comprehensive Cancer Center, University of Iowa

⁵Department of Pathology, University of Iowa

⁶Institute of Clinical and Translational Science, University of Iowa

⁷Department of Psychology and Sylvester Comprehensive Cancer Center, University of Miami

⁸Division of Gynecologic Oncology, Henry Ford Hospital

⁹Division of Gynecologic Oncology, Florida International University College of Medicine

¹⁰Department of Epidemiology, College of Public Health, University of Iowa

¹¹Department of Microbiology, University of Iowa

¹²Departments of Gynecologic Oncology and Cancer Biology, M. D. Anderson Cancer Center

¹³Division 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

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

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*Corresponding Author: Susan K. Lutgendorf, Ph.D. University of Iowa Department of Psychology E11 Seashore Hall Iowa City, IA 52242 susan-lutgendorf@uiowa.edu Phone: 319-335-2432 Fax: 319-335-0191.

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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.

Keywords

Social isolation; ovarian cancer; norepinephrine; beta-adrenergic signaling; catecholamines

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 (Shazad 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 to enhance biological processes involved in cancer progression such as angiogenesis, invasion, and metastasis (Chakroborty, et al., 2009; Sood et al., 2006; Thaker et al., 2006; Sood et al., 2010; Shazad 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; Lutgendorf et al., 2008; Nilsson et al., 2007; Sood et al., 2006; Thaker et al., 2006; Yang et al., 2002; Yang et al., 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 et al., 1994; 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 microenvironment (Costanzo et al., 2005; Lutgendorf et al., 2002; Lutgendorf et al., 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 than 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 two hours 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).

2.2.4. 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), and kept on ice prior to centrifugation, and then stored at -80°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 $20,200 \times g$ 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 mm³ 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 $\times$ 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 low concentrations (Stage I = 1.6 ± 4.56 pg/mg, Stage II = $1.04 \pm .1.20$ 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, $F(1,65) = 8.09$, $p = 0.006$. (Figure 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. (Figure 1). 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 NE was elevated in high grade compared to grade 1 and 2 tumors (281.84 ± 56.99 pg/mL vs 524.81 pg/mL ± 98.85 , $p = 0.032$) and in advanced-stage (560.66 ± 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.

3.4. Biobehavioral Factors and Catecholamines in 3 compartments

As seen in Figure 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. (Figure 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, 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 (Shazad 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), 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 microenvironment 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 minutes 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 hours 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.

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Figure 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.

Figure 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.

TABLE 1

Patient Characteristics

Measure	Cancer Patients (N = 68)
Age in years	
Mean (Standard Deviation)	59.4 (12.3)
Income	% of Patients
< 10,000	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
Marital Status	
Single	10.9
Divorced/separated	15.7
Widowed	4.7
Married/living with partner	68.7
Race	
American Indian/Alaskan Native	1.5
African American	1.5
Caucasian	97.0
Ethnicity	
Hispanic	10.3
Non-Hispanic	89.7
Stage	
I	17.6
II	7.4
III	66.2
IV	8.8
Grade	
1	11.8
2	10.3
3	77.9
Tumor Histology	
Serous	82.4
Endometrioid	13.2
Clear Cell	1.5
Other	3.0
Cytoreduction	
Optimal	77.9
Suboptimal	22.1
	mean (S.D.)

Measure	Cancer Patients (N = 68)
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)