Comprehensive Psychoneuroendocrinology 7 (2021) 100057

Contents lists available at ScienceDirect

Comprehensive Psychoneuroendocrinology

journal homepage: www.sciencedirect.com/journal/comprehensive-psychoneuroendocrinology

Tone it down: Vagal nerve activity is associated with pro-inflammatory and anti-viral factors in breast cancer – An exploratory study

Itay Ricon-Becker [a,*,2], Efrat Fogel [b,2], Steve W. Cole [c], Rita Haldar [a], Shahar Lev-Ari [d,1], Yori Gidron [e,1]

[a] School of Psychological Sciences, Sagol School of Neuroscience, Tel-Aviv University, Tel-Aviv, Israel
[b] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[c] Division of Hematology-Oncology, Department of Medicine, Department of Psychiatry & Biobehavioral Sciences, David Geffen School of Medicine at UCLA, USA
[d] Department of Health Promotion, School of Public Health, Sackler Faculty of Medicine, Tel Aviv University, Israel
[e] Faculty of Health Sciences and Welfare University of Haifa, Haifa, Israel

ARTICLE INFO

Keywords:
HRV
CTRA
Breast cancer
Perioperative
Vagal

ABSTRACT

In response to adverse social-environmental conditions, leukocytes gene expression profile is altered in a pattern recognized as the conserved transcriptional response to adversity (CTRA). This entails the up-regulated expression of pro-inflammatory genes and down-regulated expression of genes involved in type-I interferon (IFN) related anti-viral immunity. In contrast, vagal nerve activity is recognized as a significant anti-inflammatory modulator. In this work, we investigated the association between CTRA and vagal activity indicated by the standard deviation of all NN interval (SDNN), a measure of heart-rate variability, in breast cancer patients awaiting surgery (n = 16). This association was tested both at the molecular leukocyte transcription factor activity level, as well as at the cytokines serum levels. We found an association between higher SDNN and increased interferon (IFN) related anti-viral pathways, both on the leukocyte transcription factor level and serum protein level. Unexpectedly, we also found a positive correlation between higher SDNN and pro-inflammatory transcription factor activity and cytokine serum level, potentially suggesting that increased vagal activity was induced by increased inflammation, in the context of pre-surgical stress and the presence of malignant tissue. Transcription origin analysis (TOA) suggests a role for monocyte and B-cells in the anti-inflammatory and anti-metastatic effects induced by vagal nerve signaling. Larger prospective studies are needed to verify and elaborate on the results from this small cross-sectional study.

1. Introduction

All stages of tumors' progression are strongly affected by their microenvironment, including presence of cytokines, immune cells' presence and characteristics, vasculature, adjacent tissue, growth factors, inflammatory and adrenergic signaling as well as other immune related factors [1,2]. Recent research demonstrates an influence of the nervous system on tumor progression, also due to bi-directional interaction between the immune and nervous systems. An example for such an interaction is the inflammatory reflex in which the vagal nerve is a prominent component. The afferent vagal nerve conveys inflammatory signaling to the brain, while vagal efferent signaling leads to inhibition of inflammation [3,4]. Heart rate variability (HRV) is a vagal nerve activity index [5] that is based on fluctuations in the time intervals between successive heartbeats. There is a strong positive correlation (r = 0.88) between actual vagal nerve activity and HRV [6]. High HRV, indicating increased vagal activity, predicts longer cancer survival time and reduced tumor burden in human colorectal, pancreatic, prostate, lung, hepatic and breast cancer [7,8,9,10]. In contrast, inhibition of vagal activity through vagotomy has been shown to increase metastasis in some animal cancer models [11], but not in others [12].

The vagal anti-inflammatory reflex involves several routes. The pro-

* Corresponding author. Neuroimmunology Research Unit, Prof. Shamgar Ben-Eliyahu's Laboratory, School of Psychological Sciences, Tel-Aviv University, Tel-Aviv, Israel.
E-mail address: itayrico@mail.tau.ac.il (I. Ricon-Becker).
[1] Equal contributors.
[2] Equal contribution.

https://doi.org/10.1016/j.cpnec.2021.100057
Received 30 December 2020; Received in revised form 23 April 2021; Accepted 26 April 2021
Available online 30 April 2021

inflammatory cytokine interleukin-1 (IL-1) binds to IL-1 receptors in the paraganglia of the vagal nerve, which activate a cholinergic signal ascending to the brain stem. In return, pro-inflammatory cytokines (e.g. TNF, IL-6) can potentially be inhibited by two routes. First, by activating the hypothalamic pituitary adrenal (HPA) axis, resulting in inhibition of inflammation by cortisol. Second, by an efferent vagal-to-sympathetic signal to the spleen. This then stimulates a sub-group of T-cells expressing adrenergic receptors, acting as an extension of the vagal nerve in the spleen, that then secrete acetylcholine. The latter then binds to the alpha-7 nicotinic acetylcholine receptor on macrophages, which, in turn inhibits the synthesis of pro-inflammatory cytokines [13]. Overall, widespread vagal afferent signaling enables this nerve to detect tumor-associated inflammation, and to potentially regulate tumor progression via the cholinerginc anti-inflammatory reflex pathway [2].

The effects of the vagal nerve on anti-tumor and anti-viral immunity have been less studied. Vagal stimulation was shown to increase the number and activity of cytotoxic T-cells (CTLs) and to elevate the number of circulating helper T cells. Furthermore, several studies have shown that vagal activity supports anti-tumor M1 macrophage polarization via inhibiting NF-κB transcription. Also, it was shown that vagal stimulation down regulates the expression of TGF-β1, a suppressor of NK cell activity (for a review see Ref. [14]. Finally [6], showed that vagal nerve stimulation, following sepsis induction using lipopolysaccharide (LPS), preserved NK cell numbers and increased $CD8^{+}$ cells toxicity. These findings suggest that vagal activity may promote cell-mediated-immunity (CMI).

Adverse social-environmental conditions, such as social isolation and low socioeconomic status, have been associated with differential expression of gene transcripts in leukocytes and malignant tissues [15,16,17]. In leukocytes, this was defined as the conserved transcriptional response to adversity (CTRA). The CTRA includes increased expression of genes involved in inflammation (e.g. IL1B, IL8, PTGS2, TNF), and down-regulated expression of genes involved in Type-I interferon anti-viral responses (e.g. IFI-family genes) and antibody synthesis (e.g. IGJ) [18,19,20]. The Induction of CTRA activity was suggested by Cole and others (2013) to promote cancer progression [21].

Negative correlations between HRV and pro-inflammatory cytokines at the serum level have been reported in patients in general, and in breast cancer specifically [22,23],. Yet, the causal and associative relations between vagal activity and CTRA are largely unknown. As reviewed above, vagal activity exerts pro-CMI and anti-inflammatory effects, which might counteract the CTRA response. This study examined the association between vagal nerve activity indicated by the Standard deviation of all NN intervals (SDNN), a measure of HRV, and the CTRA, consisting of clusters of pro-inflammatory (e.g., IL-6, IL-8, IL-18) and anti-viral factors (e.g., INF-γ), assessed at the leukocyte transcription factor level. In order to assess the impact of potential HRV-Transcription factors (TFs) associations on cytokine levels, we also explored the association of SDNN with cytokines serum levels involved in inflammatory and anti-viral immune activity.

2. Materials and methods

The current study is a cross-sectional correlational study based on baseline data of participants from a randomized-controlled trial described in detail in Ref. [24] (NCT00502684) [25,24].

2.1. Participants

The study included a sub-group of sixteen patients with breast cancer (BC), in whom baseline electrocardiograms (ECG) were available before initiation of a pharmacological treatment in a larger study. All patients for whom an ECG was available before initiation of treatment were included in this sample. The larger study tested the effects of a beta-blocker, propranolol, plus an anti-inflammatory agent, etodolac, on perioperative immune and tumor outcomes, published by Ref. [24] (NCT00502684) [25,24]. The sixteen patients included in the current study (age 33–70, M = 54.3, SD = 8.71 years) were newly diagnosed with BC without known metastatic disease, and were recruited in three medical centers in Israel, approximately three weeks following diagnosis and 1–3 weeks before surgery. Exclusion criteria included (i) any contraindications for the drugs administered in the study, such as diabetes, asthma, cardiovascular diseases, and low blood pressure, (ii) chronic use of any beta-blocker or COX inhibitor, and (iii) a chronic autoimmune disease. Each of the three medical centers' IRB committees approved this study. All patients signed an informed consent form. See Table 1 for sample characteristics.

2.2. Instruments and procedures

Importantly, all samples and data included in this study were collected before initiation of any treatment, and specifically before initiation of propranolol and etodolac as part of the larger study.

2.2.1. Heart rate variability

Heart rate variability (HRV) is a vagal nerve activity index based on the fluctuations in the intervals between successive heartbeats. In this study, HRV analyses were based on the time domain measure of standard deviation of normal R-R intervals in milliseconds (i.e., standard deviation of normal beat-to-beat intervals, SDNN). HRV is highly correlated with actual vagal nerve activity (r = 0.88) [8]. SDNN was derived from routinely taken ECGs of approximately 10 s from participants at the medical centers. The readings were in a paper format. Digitization process and RR interval extraction were done using the CalECG software v. 4.0.0, mounting BRAVO algorithm v. 4.4.0. [AMPS llc, NY, USA]. In such brief ECG readings only time-domain parameters can be reliably obtained, and thus we used SDNN as a measure of HRV. Past studies showed that HRV from brief ECG readings are highly correlated with longer ECGs [26]. Importantly, HRV based on brief measurement was found to predict prognosis in several cancer types (For review see: [27].

2.2.2. Blood samples collection and preparation

Blood samples were taken between 7 and 11 a.m. and were transferred at room temperature to the neuroimmunology laboratory at Tel-Aviv University for processing, 2 h after blood draw. One tube of 10 ml without preservative/anticoagulant from each patient was centrifuged for 30 min at 1700g for serum collection after being allowed to clot for 2 h. A second tube was used for harvesting PBMCs from whole blood (BD Vactainer 8 ml CPT mononuclear cell preparation tube containing sodium heparin; BD bioscience; San Jose, CA, USA) according to manufacturer's instructions. PBMCs suspension was washed twice in PBS (15 min at 335g for both washes) and re-suspended in 350 μl RLT buffer (TMO, Waltham, MA, USA), in preparation for mRNA extraction. Serum samples and PBMCs in RLT were frozen at −80 °C for future analyses conducted simultaneously for all samples [24].

2.2.3. IL-6, IL-8, IL-18 and INFγ

Serum cytokines' levels were measured using enzyme linked immunosorbent assay (ELISA) according to manufacturer instruction.

Table 1
Baseline demographics of patients.

Age (Mean (range))	54.3 (33–70)	
Smoking	**No**	13
	Yes	3
Sport (0 – not at all, 1 = moderate, 2 = intensive)	**0**	5
	1	8
	2	1
	N/A	2
Inflammation/Infection in the past 2 weeks	**Yes**	3
	No	13

High-sensitivity IL-6 (HS600B), high-sensitivity IL-8 (HS800) and IL-18 (DY318), ELISA kits were purchased from R&D systems (Minneapolis, MN, USA); high-sensitivity IFNγ (BMS228HS) ELISA kits were purchased from eBioscience (San Diego, CA, USA). While IL-6, IL-8 and IL-18 are characterized predominantly as pro-inflammatory cytokines, IFNγ is characterized mainly as anti-viral and anti-tumoral.

2.2.4. CTRA

Genomics analyses used previously published reference transcriptome profiles derived from baseline PBMC samples (GSE1133) (For an elaborated description: see Ref. [25]. Briefly, total RNA was extracted from approximately 8×10^6 PBMCs, tested for suitable mass (PicoGreen RNA, Thermo-Fisher) and integrity (RNA integrity number derived from capillary electrophoresis by Agilent TapeStation) and subjected to genome-wide transcriptional profiling, using Illumina Human HT-12 v4 Expression BeadChips (Illumina Inc., San Diego, CA, USA). Genome-wide transcriptional profiles were analyzed as described below to assess activity of pro-inflammatory and Type I interferon (antiviral) transcription factor activity.

2.3. Statistical methods

2.3.1. CTRA-HRV relationships

Gene expression values were quantile-normalized, log2-transformed, and subjected to general linear model analyses to identify transcripts showing ≥1.5-fold difference in average expression over the 4-SD range of SDNN (i.e., the range spanning from 2-SD below the mean to 2-SD above it). Genes were not interpreted on an individual basis but served only as input into higher-order clusters of TELiS bioinformatics analyses [28]. Higher order transcript analysis was used to test a priori-specified hypotheses regarding CTRA-relevant transcription factors (i.e., transcription factors involved in inflammatory and anti-viral signaling). More specifically, these analyses tested whether greater HRV was associated with reduced baseline activity of pro-inflammatory transcription factors (TFs; NFκB/cRel and AP-1) and/or increased activity of Type I interferon-related TFs (IRFs), using TRANSFAC position-specific weight matrices [29] as previously described [28,25, 24]. Analyses used the prevalence of transcription factors binding motifs (TFBM) relevant for the described transcription factors among up-regulated genes as function of HRV measures, versus unregulated genes (genes which are not correlated with the SDNN measure) [30], in order to elucidate the pathways participating in creating the observed genetic regulation patterns. To confirm results from genome-wide analyses of pro-inflammatory and antiviral gene expression (which together comprise the CTRA complex), we examined the relationship between SDNN and average expression of 19 pre-specified pro-inflammatory gene transcripts (e.g., IL1B, IL6, IL8, TNF, PTGS2/Cox2) and 32 pre-specified Type I interferon response genes (e.g., IFI-, MX-, and OAS-family genes). These same pre-specified gene transcripts have been used in previous research to measure the CTRA complex [30].

Ancillary Transcript Origin Analyses (TOA) [31] were also applied to the gene sets to identify the specific leukocyte subset origins contributing to HRV-related differences in the overall leukocyte transcriptome, using cell-specific reference profiles as detailed in Refs. [32,32].

2.3.2. IL-6, IL-8, IL-18 and INFγ serum levels in relation to HRV

In order to avoid multiple statistical tests due to a small sample size we conducted an exploratory factor analysis that enabled us to detect general cytokine group patterns (factors), whose association with HRV was then tested. A varimax rotation was used and only factors with Eigen value of more than 1 were accepted. Factor scores were calculated by averaging the cytokine serum levels' Z score values within each factor and testing their correlation with HRV. There were two factors with Eigen value more than 1, the first accounted for 42.26% of variance, and the second 72.45% of variance. Both factors combined accounted for 72.45% of total variance. For all cytokines introduced into the varimax rotation see Table 2 below. A cytokine was considered not contributing to a factor if it loaded below 0.04 on to a factor. Due to the small sample size, and skewed distribution of the factors, we used spearman correlation to assess the association between SDNN and the two factors. For a full description of all SDNN and cytokine serum levels values, see Table 3 below.

Table 3: All cytokine values are provided in pg/ml. B.D. – below detection.

3. Results

3.1. The association between SDNN and serum levels of IL-6, IL-8, IL-18 and INFγ

The cytokine factor analysis yielded two factors: Factor 1 included IL-6 and IL-18 (pro-inflammatory factor) while Factor 2 included IL-8 and INFγ (combining both anti-viral and pro-inflammatory proteins). HRV (SDNN) was found to be positively correlated with Factor 2 (i.e., greater HRV was associated with increased serum presence of IFNγ and IL-8, $r = 0.64$, $p = .06$). In contrast, no correlation was found between SDNN and the pro-inflammatory factor ($r = -0.27$, $p = .31$).

3.2. The association between SDNN and CTRA expression

For HRV, 86 genes were found to be up-regulated ≥1.5 fold over a 4-SD range of variation in SDNN and 134 were down-regulated ≥1.5 fold. TELiS bioinformatics analyses linked greater SDNN to greater activation of multiple CTRA-related transcription control pathways, including greater activity of the pro-inflammatory factors, AP-1 (log2 TFBM ratio in up-vs down-regulated promoters: 1.37 ±0 .27 $p < .0001$) and NF-κB (0.88 ± 0.32, $p = .007$), as well as greater activity of Interferon Regulatory Factors (IRF) (1.18 ± 0.40, $p = .004$) (Fig. 1). Follow-up analyses using an alternative assessment of CTRA gene expression based on a priori-specified gene sets showed no significant association of SDNN with average expression of 19 pro-inflammatory gene transcripts (−0.047 log2 mRNA abundance per SD in SDNN ± 0.056 SE, $p = .407$) but a significant up-regulation of 32 Type-I interferon response genes (+0.103 ± 0.041, $p = .019$).

Fig. 1: The Association between greater SDNN and AP-1, NF-kB, and IRF transcription factors.

3.3. TOA (transcript origin analysis)

TOA analyses of cellular origin, using the HT12 reference data on PBMC subsets, implicated monocytes and B cells as major sources of differential gene expression (CD14 Monocytes $p = .0172$, CD19 BCells $p = .0003$).

4. Discussion

High vagal activity may inhibit cancer progression via its anti-inflammatory and anti-viral effects [33,2,14,34] In contrast CTRA is characterized by up-regulated expression of pro-inflammatory genes and down-regulated anti-viral genes, and, as can be expected, was associated with increased cancer progression [21]. We studied the relationship between vagal nerve activity (measured by SDNN), and

Table 2
Cytokines introduced into the factor analysis and their correlations to each of the two factors.

Cytokine	Factor 1	Factor 2
IL-6	**.901**	-.059
IL-8	-.225	**.828**
IL-18	**.847**	.1
IFN-γ	.315	**.72**

Table 3
SDNN values and Cytokine serum levels (pg/ml).

Patient #	SDNN	IL-6 (pg/ml)	IL-8 (pg/ml)	IL18 (pg/ml)	IFN-g (pg/ml)
1	22.05	1.05	19.11	326.39	B.D
2	12.33	.62	13.19	162.94	.04
3	43.84	2.76	16.82	259.92	2.33
4	19.21	2.12	12.90	178.04	B.D
5	12.65	.88	41.15	371.24	B.D
6	27.13	32.31	17.23	170.70	B.D
7	27.08	2.75	13.43	352.76	.44
8	25.49	2.20	17.48	335.74	B.D
9	8.97	5.64	24.62	348.83	B.D
10	51.84	1.09	21.78	216.37	.23
11	24.85	.74	15.58	369.15	B.D
12	19.21	.92	16.97	254.28	.16
13	33.33	1.79	11.80	248.39	.35
14	22.76	6.21	14.69	314.33	.29
15	19.15	2.22	10.20	146.23	.32
16	57.28	.65	16.24	189.56	.23

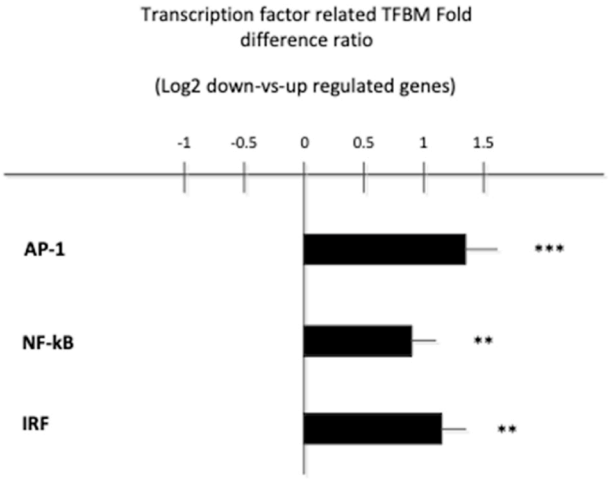

Fig. 1. The association between the HRV measure SDNN and AP-1, NF-kB, and IRF related TFBMs prevalence in down vs. upregulated promoter genes associated with HRV. Significance is indicated by * ($p < .05$), **($p < .01$), ***($p < .0001$).

CTRA, within breast cancer patients awaiting surgery. As would be predicted from the above, SDNN was positively associated with the IFN component of CTRA. Unexpectedly, SDNN was also positively related to the pro-inflammatory NF-kB and general immune activation AP-1 pathways. Finally, regarding serum cytokine levels, SDNN showed a trend towards a positive correlation with higher circulating levels of IFN-γ and IL-8, reflecting the same pattern evident at the transcription factor level. Importantly, the positive correlation between pro-inflammatory markers and HRV contradicts a recent meta-analysis of 151 studies showing a negative correlation between HRV and inflammatory markers [23].

The association between greater vagal activity (higher SDNN) and general immune pathways that include pro-inflammatory transcription factors (NF-κB and AP-1) and serum pro-inflammatory cytokine IL-8 levels was somewhat unexpected, given existing literature describing vagal anti-inflammatory effects. Keeping the cross-sectional design of the present study in mind, a possible explanation may be the reported induction of NFkB by IFN, in an alternative NIK and TRAF dependent pathway [35], that are involved in processing and in regulating inflammatory signals. Additionally, the current findings are derived from a cross-sectional study, and higher HRV may have reflected vagal afferent responses to inflammation (e.g., high IL-8), rather than vagal regulatory efferent responses. It is also important to note that these data were collected from cancer patients awaiting surgery, an event known to exhibit heightened levels of psychological stress. Thus, these findings may be affected by interactions with stress and the presence of the malignant tissue with its potential pro-inflammatory and immune suppressive effects. Finally, it is important to note that RNA analyses of a broad set of 19 pro-inflammatory gene transcripts did not find broad inflammatory up-regulation, which suggests that the inflammatory effects observed here at both the transcription factor and serum cytokine level may be restricted in scope and potentially secondary to the increased antiviral activity that was consistently documented across all measures.

HRV showed a positive association with the anti-viral component of CTRA. This suggests that the vagal nerve regulates anti-viral responses. This is consistent with vagal activity being positively associated with inhibition of TGF-β1, a suppressing factor of NK-cells [36,37,38,39], thus potentially enabling greater anti-cancer activity.

The factor analysis grouped INFγ and IL-8 on one factor, showing a trend for a positive correlation with HRV, implying that both cytokines share a latent factor. When NK cells are induced to secrete IFNγ, they produce IL-8 which then in return facilitates INFγ production [40]. Additionally, IL-8 was also found to have an important role in host responses to infection [41]. The relationship of IFNγ and IL-8 to anti-viral properties might account for their mutual correlation with SDNN, and may support our findings at the transcription factor level, showing a positive association between SDNN and the anti-viral branch of the CTRA construct.

Notably, the positive association between HRV and the anti-viral component of CTRA was specific to two interferon regulatory factors (IRFs), IRF1 and IRF2. IRF2 inhibits Type-I interferon response, which facilitate a potent anti-viral type-II interferon response (i.e., IFN-γ secretion). Importantly, downstream effects of IRF1 activate IFN type I and II responses, as well as activates NF-kB, leading both to activation of anti-viral and also to pro-inflammatory responses [42]. This combined IRF1 and IRF2 association with vagal activity might also account for the unexpected and complex results of our study.

TOA indicated that monocytes and B cells were the sources of differential gene expression associated with HRV (SDNN). This may implicate M1 type macrophages in the association between HRV and anti-viral immunity. IFN-γ, associated here with HRV, is the main cytokine associated with M1 polarization, and macrophages themselves produce IFN-γ [43]. M1 macrophages have potent antitumor activity directly through killing of cancer cells and inhibition of angiogenesis, and indirectly through stimulation of T cells and Th1 [44,45] Whether high HRV predicts cancer favorable prognosis [46] via this M1-macrophages related pathway needs to be tested in prospective studies.

Our findings may suggest another mechanism linking vagal activity with the CTRA complex and cancer prognosis. Monocytes and B cells are antigen presenting cells (APC) [47]. Tumor antigen presentation by monocytes' MHC is crucial, as tumors escape T-cell immune surveillance by reducing their MHC-I expression [48]. INFγ facilitates re-expression of MHC-I molecules on tumors, including in human cancers [49,50], which is critical in the induction of tumor recognition and rejection by T-cells. Thus, promoting MHC-I expression via IFNγ on monocytes and B-cells may be a mechanism via which vagal activity promotes better cancer prognosis related to CTRA genes.

This small pilot study suffered a number of limitations: small sample size, and a cross sectional design restricted to women currently diagnosed with breast cancer. Furthermore, the mechanisms proposed here could not be examined. In particular, this sample size was too small to allow statistical control for differences in leukocyte subset abundance, which might confound direct effects of the vagal nerve on inflammatory gene regulation. For example, vagal activity may have induced increased trafficking of NK cells, non-classical monocytes, or other leukocyte subsets, that harbor elevated levels of pro-inflammatory transcription factor activity. Additionally, conducting an ECG might

induce stress by itself. Future studies should aim to control for stress elicited by the ECG itself to accurately assess if effects such as those shown herein are due to an acute response to stress or represent tonic levels of vagal activity.

In conclusion, further research with a prospective design, larger samples, and leukocyte subset isolation, is needed, and may shed light on possible new anti-tumoral mechanisms explaining potential pathways by which high vagal activity predicts better cancer prognosis [46]. Furthermore, interventions testing the effects of vagal activation on cancer may wish to also include inflammatory and anti-viral markers to shed a light on mechanisms on any therapeutic effects.

Author contributions

Itay Ricon- Becker & Efrat Fogel: Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Steve W. Cole: Conceptualization; Formal analysis; Investigation; Methodology; Software; Visualization; Writing - review & editing. Rita Haldar: Data curation; Investigation; Resources; Writing - review & editing. Shahar Lev-Ari & Yori Gidron: Conceptualization; Formal analysis; Methodology; Project administration; Supervision; Roles/Writing - original draft; Writing - review & editing.

Funding

This work was supported by the National Cancer Institute Network on Biobehavioral Pathways in Cancer (grant number 13XS084; to S. Ben-Eliyahu); the Israel Science Foundation (grant 1406/10; to S. Ben-Eliyahu); and the National Institutes of Health/National Institute on Aging (grant P30 AG017265; to S. Cole).

Declaration of competing interest

None.

References

[1] S.S. Chavan, V.A. Pavlov, K.J. Tracey, Mechanisms and therapeutic relevance of neuro-immune communication, Immunity (2017), https://doi.org/10.1016/j.immuni.2017.06.008.

[2] Y. Gidron, H. Perry, M. Glennie, Does the vagus nerve inform the brain about preclinical tumours and modulate them? Lancet Oncol. (2005) https://doi.org/10.1016/S1470-2045(05)70096-6.

[3] V.A. Pavlov, K.J. Tracey, The vagus nerve and the inflammatory reflex-linking immunity and metabolism, Nat. Publ. Gr. 8 (2012) 743–754, https://doi.org/10.1038/nrendo.2012.189.

[4] K.J. Tracey, Reflex control of immunity, Nat. Rev. Immunol. (2009), https://doi.org/10.1038/nri2566.

[5] T.B.J. KUO, C.J. LAI, Y.-T. HUANG, C.C.H. YANG, Regression analysis between heart rate variability and baroreflex-related vagus nerve activity in rats, J. Cardiovasc. Electrophysiol. 16 (2005) 864–869, https://doi.org/10.1111/j.1540-8167.2005.40656.x.

[6] S. Mihaylova, H. Schweighöfer, H. Hackstein, B. Rosengarten, Effects of anti-inflammatory vagus nerve stimulation in endotoxemic rats on blood and spleen lymphocyte subsets, Inflamm. Res. 63 (2014) 683–690, https://doi.org/10.1007/s00011-014-0741-5.

[7] J.K. Chiang, M. Koo, T.B.J. Kuo, C.H. Fu, Association between cardiovascular autonomic functions and time to death in patients with terminal hepatocellular carcinoma, J. Pain Symptom Manag. 39 (2010) 673–679, https://doi.org/10.1016/j.jpainsymman.2009.09.014.

[8] J. Hayano, Y. Sakakibara, A. Yamada, M. Yamada, S. Mukai, T. Fujinami, K. Yokoyama, Y. Watanabe, K. Takata, Accuracy of assessment of cardiac vagal tone by heart rate variability in normal subjects, Am. J. Cardiol. 67 (1991) 199–204, https://doi.org/10.1016/0002-9149(91)90445-Q.

[9] C. Mouton, A. Ronson, D. Razavi, F. Delhaye, N. Kupper, M. Paesmans, M. Moreau, J.M. Nogaret, A. Hendlisz, Y. Gidron, The relationship between heart rate variability and time-course of carcinoembryonic antigen in colorectal cancer, Auton. Neurosci. Basic Clin. 166 (2012) 96–99, https://doi.org/10.1016/j.autneu.2011.10.002.

[10] Y.M. Wang, H.T. Wu, E.Y. Huang, Y.R. Kou, S.S. Hseu, Heart rate variability is associated with survival in patients with brain metastasis: a preliminary report, BioMed Res. Int. (2013), https://doi.org/10.1155/2013/503421.

[11] L.I. Partecke, A. Käding, D.N. Trung, S. Diedrich, M. Sendler, F. Weiss, J.P. Kühn, J. Mayerle, K. Beyer, W. von Bernstorff, C.D. Heidecke, W. Keßler, Subdiaphragmatic vagotomy promotes tumor growth and reduces survival via TNFα in a murine pancreatic cancer model, Oncotarget 8 (2017) 22501–22512, https://doi.org/10.18632/oncotarget.15019.

[12] A. Novotny, K. Ryberg, J. Heiman Ullmark, L. Nilsson, A. Khorram-Manesh, S. Nordgren, D.S. Delbro, G. Nylund, Is acetylcholine a signaling molecule for human colon cancer progression? Scand. J. Gastroenterol. 46 (2011) 446–455, https://doi.org/10.3109/00365521.2010.539252.

[13] M. Rosas-Ballina, P.S. Olofsson, M. Ochani, S.I. Valdés-Ferrer, Y.A. Levine, C. Reardon, M.W. Tusche, V.A. Pavlov, U. Andersson, S. Chavan, T.W. Mak, K. J. Tracey, Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit, Science 334 (2011) 98–101, https://doi.org/10.1126/science.1209985.

[14] E. Reijmen, L. Vannucci, M. De Couck, J. De Grève, Y. Gidron, Therapeutic potential of the vagus nerve in cancer, Immunol. Lett. (2018), https://doi.org/10.1016/j.imlet.2018.07.006.

[15] S.W. Cole, Human social genomics, PLoS Genet. (2014), https://doi.org/10.1371/journal.pgen.1004601.

[16] S.W. Cole, G. Conti, J.M.G. Arevalo, A.M. Ruggiero, J.J. Heckman, S.J. Suomi, Transcriptional modulation of the developing immune system by early life social adversity, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 20578–20583, https://doi.org/10.1073/pnas.1218253109.

[17] S.W. Cole, L.C. Hawkley, J.M. Arevalo, C.Y. Sung, R.M. Rose, J.T. Cacioppo, Social regulation of gene expression in human leukocytes, Genome Biol. 8 (2007) 1–13, https://doi.org/10.1186/gb-2007-8-9-r189.

[18] S.W. Cole, J.M.G. Arevalo, R. Takahashi, E.K. Sloan, S.K. Lutgendorf, A.K. Sood, J. F. Sheridan, T.E. Seeman, Computational identification of gene-social environment interaction at the human IL6 locus, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 5681–5686, https://doi.org/10.1073/pnas.0911515107.

[19] G.E. Miller, E. Chen, A.K. Fok, H. Walker, A. Lim, E.F. Nicholls, S. Cole, M.S. Kobor, Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 14716–14721, https://doi.org/10.1073/pnas.0902971106.

[20] G.E. Miller, E. Chen, J. Sze, T. Marin, J.M.G. Arevalo, R. Doll, R. Ma, S.W. Cole, A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-κB signaling, Biol. Psychiatr. 64 (2008) 266–272, https://doi.org/10.1016/j.biopsych.2008.03.017.

[21] S.W. Cole, Nervous system regulation of the cancer genome, Brain Behav. Immun. 30 (2013) S10–S18, https://doi.org/10.1016/j.bbi.2012.11.008.

[22] A.D. Crosswell, K.G. Lockwood, P.A. Ganz, J.E. Bower, Low heart rate variability and cancer-related fatigue in breast cancer survivors, Psychoneuroendocrinology 45 (2014) 58–66, https://doi.org/10.1016/j.psyneuen.2014.03.011.

[23] D.W.P. Williams, J. Koenig, L. Carnevali, A. Sgoifo, M.N. Jarczok, E.M. Sternberg, J.F. Thayer, Heart rate variability and inflammation: a meta-analysis of human studies, Brain Behav. Immun. 80 (2019) 219–226, https://doi.org/10.1016/j.bbi.2019.03.009.

[24] L. Shaashua, M. Shabat-Simon, R. Haldar, P. Matzner, O. Zmora, M. Shabtai, E. Sharon, T. Allweis, I. Barshack, L. Hayman, J. Arevalo, J. Ma, M. Horowitz, S. Cole, S. Ben-Eliyahu, Perioperative COX-2 and β-adrenergic blockade improves metastatic biomarkers in breast cancer patients in a phase-II randomized trial, Clin. Canc. Res. 23 (2017) 4651–4661, https://doi.org/10.1158/1078-0432.CCR-17-0152.

[25] R. Haldar, L. Shaashua, H. Lavon, Y.A. Lyons, O. Zmora, E. Sharon, Y. Birnbaum, T. Allweis, A.K. Sood, I. Barshack, S. Cole, S. Ben-Eliyahu, Perioperative inhibition of β-adrenergic and COX2 signaling in a clinical trial in breast cancer patients improves tumor Ki-67 expression, serum cytokine levels, and PBMCs transcriptome, Brain Behav. Immun. 73 (2018) 294–309, https://doi.org/10.1016/j.bbi.2018.05.014.

[26] R.M. Hamilton, P.S. Mckechnie, P.W. Macfarlane, Can cardiac vagal tone be estimated from the 10-second ECG? Int. J. Cardiol. 95 (2004) 109–115, https://doi.org/10.1016/j.ijcard.2003.07.005.

[27] M. De Couck, R. Caers, D. Spiegel, Y. Gidron, The role of the vagus nerve in cancer prognosis: a systematic and a comprehensive review, J. Oncol. (2018), https://doi.org/10.1155/2018/1236787.

[28] S.W. Cole, W. Yan, Z. Galic, J. Arevalo, J.A. Zack, Expression-based monitoring of transcription factor activity: the TELiS database, Bioinformatics 21 (2005) 803–810, https://doi.org/10.1093/bioinformatics/bti038.

[29] H. Karas, R. Knüppel, W. Schulz, H. Sklenar, E. Wingender, Combining structural analysis of DNA with search routines for the detection of transcription regulatory elements, Bioinformatics 12 (1996) 441–446, https://doi.org/10.1093/bioinformatics/12.5.441.

[30] S.W. Cole, The conserved transcriptional response to adversity, Curr. Opin. Behav. Sci. (2019), https://doi.org/10.1016/j.cobeha.2019.01.008.

[31] S.W. Cole, L.C. Hawkley, J.M.G. Arevalo, J.T. Cacioppo, Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 3080–3085, https://doi.org/10.1073/pnas.1014218108.

[32] D.S. Black, S.W. Cole, G. Christodoulou, J.C. Figueiredo, Genomic mechanisms of fatigue in survivors of colorectal cancer, Cancer 124 (2018) 2637–2644, https://doi.org/10.1002/cncr.31356.

[33] M. De Couck, R. Maréchal, S. Moorthamers, J.L. Van Laethem, Y. Gidron, Vagal nerve activity predicts overall survival in metastatic pancreatic cancer, mediated by inflammation, Cancer Epidemiol 40 (2016) 47–51, https://doi.org/10.1016/j.canep.2015.11.007.

[34] K.J. Tracey, Physiology and immunology of the cholinergic antiinflammatory pathway, J. Clin. Invest. (2007), https://doi.org/10.1172/JCI30555.

[35] L.M. Pfeffer, The role of nuclear factor κb in the interferon response, J. Interferon Cytokine Res. (2011), https://doi.org/10.1089/jir.2011.0028.
[36] F. Ghiringhelli, C. Ménard, M. Terme, C. Flament, J. Taieb, N. Chaput, P.E. Puig, S. Novault, B. Escudier, E. Vivier, A. Lecesne, C. Robert, J.Y. Blay, J. Bernard, S. Caillat-Zucman, A. Freitas, T. Tursz, O. Wagner-Ballon, C. Capron, W. Vainchencker, F. Martin, L. Zitvogel, CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-β-dependent manner, J. Exp. Med. 202 (2005) 1075–1085, https://doi.org/10.1084/jem.20051511.
[37] Y. Lu, J.-J. Liu, X.-Y. Bi, X.-J. Yu, S.-S. Kong, F.-F. Qin, J. Zhou, W.-J. Zang, Pyridostigmine ameliorates cardiac remodeling induced by myocardial infarction via inhibition of the transforming growth factor-β1/TGF-β1–activated kinase pathway, J. Cardiovasc. Pharmacol. 63 (2014) 412–420, https://doi.org/10.1097/FJC.0000000000000062.
[38] S. Viel, A. Marçais, F.S.F. Guimaraes, R. Loftus, J. Rabilloud, M. Grau, S. Degouve, S. Djebali, A. Sanlaville, E. Charrier, J. Bienvenu, J.C. Marie, C. Caux, J. Marvel, L. Town, N.D. Huntington, L. Bartholin, D. Finlay, M.J. Smyth, T. Walzer, TGF-β inhibits the activation and functions of NK cells by repressing the mTOR pathway, Sci. Signal. 9 (2016), ra19–ra19, https://doi.org/10.1126/scisignal.aad1884.
[39] Z. Wang, L. Yu, B. Huang, S. Wang, K. Liao, G. Saren, X. Zhou, H. Jiang, Low-level transcutaneous electrical stimulation of the auricular branch of vagus nerve ameliorates left ventricular remodeling and dysfunction by downregulation of matrix metalloproteinase 9 and transforming growth factor β1, J. Cardiovasc. Pharmacol. 65 (2015) 342–348, https://doi.org/10.1097/FJC.0000000000000201.
[40] S.M. Poznanski, A.J. Lee, T. Nham, E. Lusty, M.J. Larché, D.A. Lee, A.A. Ashkar, Combined stimulation with interleukin-18 and interleukin-12 potently induces interleukin-8 production by natural killer cells, J. Innate Immun. 9 (2017) 511–525, https://doi.org/10.1159/000477172.
[41] L. Del Rio, S. Bennouna, J. Salinas, E.Y. Denkers, CXCR2 deficiency confers impaired neutrophil recruitment and increased susceptibility during toxoplasma gondii infection, J. Immunol. 167 (2001) 6503–6509, https://doi.org/10.4049/jimmunol.167.11.6503.
[42] H. Negishi, T. Taniguchi, H. Yanai, The Interferon (IFN) Class of Cytokines and the IFN Regulatory Factor (IRF) Transcription Factor Family, 2018, https://doi.org/10.1101/cshperspect.a028423.
[43] C.D. Mills, K. Kincaid, J.M. Alt, M.J. Heilman, A.M. Hill, M-1/M-2 macrophages and the Th1/Th2 paradigm, J. Immunol. 164 (2000) 6166–6173, https://doi.org/10.4049/jimmunol.164.12.6166.
[44] G.L. Beatty, E.G. Chiorean, M.P. Fishman, B. Saboury, U.R. Teitelbaum, W. Sun, R. D. Huhn, W. Song, D. Li, L.L. Sharp, D.A. Torigian, P.J. O'Dwyer, R. H. Vonderheide, CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans, Science 331 (2011) 1612–1616, https://doi.org/10.1126/science.1198443.
[45] O.A.W. Haabeth, B. Bogen, A. Corthay, A model for cancer-suppressive inflammation, OncoImmunology 1 (2012) 1146–1155, https://doi.org/10.4161/onci.21542.
[46] X. Zhou, Z. Ma, L. Zhang, S. Zhou, J. Wang, B. Wang, W. Fu, Heart rate variability in the prediction of survival in patients with cancer: a systematic review and meta-analysis, J. Psychosom. Res. (2016), https://doi.org/10.1016/j.jpsychores.2016.08.004.
[47] R.A.M. Rossetti, N.P.C. Lorenzi, K. Yokochi, M.B.S. de F. Rosa, L. Benevides, P.F. R. Margarido, E.C. Baracat, J.P. Carvalho, L.L. Villa, A.P. Lepique, B lymphocytes can be activated to act as antigen presenting cells to promote anti-tumor responses, PloS One 13 (2018), e0199034, https://doi.org/10.1371/journal.pone.0199034.
[48] J. Bubeník, MHC class I down-regulation: tumour escape from immune surveillance? (review), Int. J. Oncol. (2004), https://doi.org/10.3892/ijo.25.2.487.
[49] Y. Gidron, L. Vannucci, Playing in three makes it simpler: mapping the cognitive figure-ground framework onto cancer-immunology and immunotherapy (Review), Int. J. Oncol. 36 (2010) 1061–1065, https://doi.org/10.3892/ijo_00000587.
[50] I. Yang, T.J. Kremen, A.J. Giovannone, E. Paik, S.K. Odesa, R.M. Prins, L.M. Liau, Modulation of major histocompatibility complex Class I molecules and major histocompatibility complex-bound immunogenic peptides induced by interferon-α and interferon-γ treatment of human glioblastoma multiforme, J. Neurosurg. 100 (2004) 310–319, https://doi.org/10.3171/jns.2004.100.2.0310.