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B=Basal Cell Carcinoma: Stressful Life Events and the Tumor Environment

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Abstract

Context—Child emotional maltreatment can result in lasting immune dysregulation that may be heightened in the context of more recent life stress. Basal cell carcinoma (BCC) is the most common skin cancer, and the immune system plays a prominent role in tumor appearance and progression.

Objective—To address relationships among recent severe life events, childhood parental emotional maltreatment, depression, and messenger RNA (mRNA) coding for immune markers associated with BCC tumor progression/regression.

Setting—University medical center

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The corresponding author had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Design—We collected information about early parent-child experiences, severe life events in the last year as assessed by the Life Events and Difficulties Schedule (LEDS), depression, and mRNA for immune markers associated with BCC tumor progression/regression from BCC tumor patients.

Participants—91 BCC patients (ages 23–92) who had all had a previous BCC tumor.

Main Outcome Measures—The expression of four BCC tumor mRNA markers (CD25, CD3ε, ICAM-1, and CD68) that have been linked to BCC tumor progression/regression were assessed in BCC tumor biopsies.

Results—Both maternal and paternal emotional maltreatment interacted with the occurrence of severe life events to predict the local immune response to the tumor (adjusted p=0.009, p=0.03, respectively). Among BCC patients who had experienced a severe life event within the past year, those who were emotionally maltreated by their mothers (p=0.007) or fathers (p=0.02) as children had a poorer immune response to the BCC tumor. Emotional maltreatment was unrelated to BCC immune responses among those who did not experience a severe life event. Depressive symptoms were not associated with the local tumor immune response.

Conclusions—Troubled early parent-child relationships, in combination with a severe life event in the past year, predicted immune responses to a BCC tumor. The immunoreactivity observed in BCCs and the surrounding stroma reflects an anti-tumor-specific immune response that can be altered by stress.

Stressful events and the negative emotions they generate can dysregulate immunity sufficient to produce clinically significant alterations.[1] Acute and chronic stressors can impair vaccine responses, slow wound healing, promote inflammation, and dampen markers of both innate and adaptive immune function.[2–6] Those who experienced adverse childhood events are particularly sensitive to subsequent stressors.[7–10]

Converging evidence suggests that highly stressful events early in life can have long-term consequences for immune system regulation. Childhood maltreatment has been associated with elevated inflammation and higher antibody titers to herpes simplex virus type 1 (reflecting poorer cellular immune function).[11–15] Child maltreatment has also been linked to multiple diseases, including cancer; immune dysregulation likely contributes to these effects.[16]

Skin cancer, the most common cancer in the United States, is more prevalent than all other malignancies combined.[18, 19] The incidence of basal cell carcinoma (BCC), the most common skin cancer, has been doubling every 14 years.[20] The risk for subsequent BCCs after an initial tumor is substantial, with 44% developing additional lesions within three years.[21] Risk factors for the first or index BCC include age, childhood sun exposure, fair skin, and being male; however, subsequent tumors are not reliably related to these variables.[21, 22]

The immune system plays a prominent role in BCC tumor appearance and progression.[23] A significant increase in the expression of CD3ε (total T cells) and CD25 (IL-2 receptor) has been observed in actively regressing tumors compared with those showing no current or past regression.[24] Furthermore, increased expression of ICAM-1 and infiltration of CD68+ cells (macrophages) have been described during BCC tumor regression after treatment with imiquimod (a topical cream that enhances the local immune response against BCCs).[25, 26] Histological evaluations of excised BCCs show that 50% provide evidence of at least partial regression. The immunoreactivity observed in BCCs and the surrounding stroma reflects an anti-tumor-specific immune response.[23]

Immunosuppressive treatments clearly increase BCC incidence. Organ transplant recipients have a ten-fold risk compared to the general population.[27] However, even much milder alterations in cell-mediated immunity can be consequential. For example, oral glucocorticoid therapy boosts BCC incidence, likely through decreased immunosurveillance.[28, 29]

Chronic stressors can be a powerful immunomodulator during critical developmental periods, setting the stage for future alterations in skin cancer tumors. Mice that had been subjected to restraint stress subsequently developed UV-induced squamous cell carcinoma more rapidly than non-stressed control mice.[30] Furthermore, stressed mice also had a poorer immune response as assessed by messenger RNA (mRNA) immune markers in their tumors compared to controls. Indeed, even well after the stressor ended, the tumors of stressed mice did not regress like those of controls, suggesting that stressors early in development can continue to influence the immune response long after stress exposure.

Early stressful experiences combined with subsequent stress may be particularly detrimental. Seligman and colleagues exposed young rats to inescapable, escapable, or no shock conditions.[17] When these rats reached adulthood, they were injected with cancer cells and exposed to one of the three shock conditions again. Rats exposed to inescapable shock when young (i.e., an early environmental stressor) were more likely to develop tumors if also exposed to either shock condition as adults. However, rats that did not experience inescapable shock when they were young were not more likely to develop a tumor when exposed to shock as adults. These data suggest early life stressors may alter the immune response to subsequent stressors, thus decreasing anti-tumor defenses.

Parental emotional maltreatment is a common stressor in childhood. Maltreatment in childhood has been associated with atypical cortisol production throughout the day.[31, 32] In adults, troubled early parent-child relationships have been linked to more pronounced stress-induced glucocorticoid production.[33, 34] Compared to those who had healthy parent-child relationships, those who had adverse parent-child relationships are more likely to have emotional difficulties when they encounter subsequent stressors.[35]

Accordingly, this study addressed how parental emotional maltreatment and subsequent stressors might impact the BCC tumor environment. Assessment of the BCC tumor environment included four mRNA markers (i.e. CD25, CD3ε, ICAM-1, and CD68) that have been linked to BCC tumor progression/regression. BCC tumor biopsies were taken from excised tissue. We hypothesized that childhood parental emotional maltreatment would be associated with a poorer local immune response to a BCC tumor, if accompanied by a recent life stressor.

Methods

Participants

Patients who had a newly diagnosed, histologically verified BCC received a letter from their treating dermatologist that described the study. Disqualifying health problems included immunosuppressive therapies or immunological treatments for other medical conditions, another cancer diagnosed within the last five years (except for a prior BCC), or any history of squamous cell carcinoma (SCC) or melanoma. We assessed childhood experiences and mRNA immune markers associated with BCC tumor progression/regression in 91 participants who all had at least one prior BCC tumor. We chose to study individuals who previously had a BCC tumor because we wanted to know what sets the stage for subsequent BCC tumors that develop within three years in almost half of the people following their BCC.[21] The presence of the first tumor indicates that people already had enough sun

exposure to get a BCC tumor, and met their personal age threshold to start developing them. Accordingly, by studying individuals who had a prior history, we had a stronger basis for investigating psychosocial influences. This study was approved by the local institutional review board, and participants provided written informed consent.

RNA extraction and cDNA synthesis

We examined the expression of four mRNA markers, CD25 (the alpha chain of the interleukin (IL)-2 receptor expressed on activated T-cells and B-cells), CD3ε (a T-cell receptor marker), ICAM-1 (a cell surface glycoprotein on endothelial cells and immune cells), and CD68 (a marker for monocytes/macrophages) in BCC tumor tissue. The mRNA markers encode for proteins that are expressed on various immune cells; CD25 is the alpha chain of the interleukin (IL)-2 receptor expressed on activated T-cells and B-cells, CD3ε is part of the T-cell receptor-CD3 complex that has an important role in signal transduction after antigen recognition by the T-cell, ICAM-1 is a cell surface glycoprotein on endothelial cells and immune cells that functions in the endothelial transmigration of immune cells, and CD68 is a hematopoietic differentiation marker of the monocyte/macrophage lineage.

Four 10 μm thick sections obtained from each paraffin-embedded diagnostic biopsy were immediately deparaffinized with xylene followed by hydration through graded ethanol washes. The tissue was then centrifuged and digested with 100 μl digestion buffer (0.01M Tris pH7.8, 0.005M EDTA, and 0.5% SDS) plus 100 μl of 20 mg/ml proteinase K for 24 hours at 55°C with agitation. Total RNA was extracted by adding 800 μl Trizol Reagent (Life Technologies) with 200 μg/ml glycogen as a carrier. Total RNA was precipitated with 650 μl of 100% isoproponol at −40°C overnight then centrifuged at maximum speed at room temperature. The pellets were washed twice with 75% ice-cold ethanol. The RNA pellet was resuspended in nuclease-free H_2O, and quantified using a Nanodrop spectrophotometer. Total RNA (1 μg) was treated with DNase I (Life Technologies), followed by cDNA synthesis using Superscript III RNase H- reverse transcriptase (Life Technologies). The cDNA was stored at −80°C until used for real-time PCR.

Real-time PCR

TaqMan Gene Expression Assays (Applied Biosystems) were used for both internal positive controls and the genes of interest. Using the Taqman Human Endogenous Control Plates (Applied Biosystems) and the GeNorm software (http://medgen.ugent.be/~jvdesomp/genorm/) we selected the following as best-fit internal positive controls for this study: GAPDH (glyceraldehydes-3-phosphate-dehydrogenase; Assay ID: 4326317E) and RPLP0 (large ribosomal protein; Assay ID 4326314E). The expression of CD3ε (Assay ID: Hs99999153_m1), CD25 (Assay ID: 4328847F), CD68 (Assay ID: Hs00154355_m1), and ICAM-1 (Assay ID: Hs99999152_m1) mRNAs were normalized to the geometric average of the C_Ts for GAPDH and RPLP0. TaqMan Gene Expression Assay Reagents do not detect genomic DNA sequences; therefore, these are specific to mRNA. The mRNA levels between samples were compared using relative real-time PCR with TaqMan fluorogenic probes, TaqMan PCR Reagent Kit and 7300 Real-Time PCR System (Applied Biosystems). Since the complete study involved running samples in several 96-well plates, one cDNA sample from leukocytes previously characterized to express the genes of interest was also included in all of the PCR runs to serve as the plate control for normalization. All the genes of interest were first normalized to the internal positive control, then normalized to the plate control, and the relative expression of mRNA species was calculated using the comparative C_T method as described by the manufacturer (see User bulletin #2 Applied Biosystems, P/N 4303859, 1997).[36]

Psychological and Health Related Measures

Detailed life event interviews were conducted to assess severe life events in the past year. The Life Events and Difficulty Schedule (LEDS) interview assesses the occurrence of over 200 stressful events, with a goal of understanding the environmental stressors, while avoiding some of the biases that could occur from emotional status or coping resources.[37] When events are identified, interviewers gather data about a set of contextual factors that might intensify the meaning and implications of the event. For example, loss of employment is likely more stressful for someone who is already in debt and the sole family provider than one who has alternate sources of income. The LEDS has been widely used in psychiatric research, and severe life events predict the occurrence of psychiatric disorders, particularly depression. There are also documented links between the LEDS and illnesses.[38] For example, life stressors assessed by the LEDS were linked to increased cold susceptibility.[39]

Data gathered during interviews were presented to a set of raters who were blind to the participant's emotional or subjective reaction to the life stressor, SCID data, child maltreatment scores, BCC mRNA levels, and participant's health. Raters, who were trained by S. Johnson, judged the severity of each life event on a five-point scale ranging from 1 (marked) to 5 (little or no) negativity using the Bedford College dictionaries, which provide example ratings for over 1,000 life events occurring in different life contexts. They also rated whether events might be related to BCC status (e.g., medical complications, lifestyle changes, or other ways in which the cancer diagnosis may have provoked the stressor). Any discrepancies were resolved through group discussion and consensus ratings. Inter-rater reliability was evaluated each month, and was sustained at a level above intraclass correlations of .80 throughout the study. Inconsistencies across raters were reviewed, and ongoing training was conducted to ensure that raters followed guidelines carefully. Our study focused on the presence of at least one severe life event (as defined by severity ratings of 1 or 2) that was independent of the BCC and other illnesses. Although severe events can differ across individuals, the most common severe life events include the loss of a core confidant relationship, death of a family member, marital separation or divorce, or the loss of employment for the primary wage earner in the family. Because our goal was to document that environmental stressors can predict changes in disease status, we focused on events that were not related to illness to ensure that we were capturing stressors that were unrelated to an underlying biological vulnerability.

Although the LEDS takes approximately 10 hours for interviews, transcribing, and rating, the instrument's well-documented validity suggest that this care is warranted. It has significantly higher reliability and validity compared with self-report measures.[37, 38, 40, 41]

The depression module of the Structured Clinical Interview for DSM-IV, nonpatient version (SCID-NP) was administered by clinically-trained interviewers to make relatively rapid and valid DSM-IV diagnoses.[42] Inter-rater reliability for SCID-NP diagnoses were calculated using randomly selected audiotapes for 20% of the participants. There was greater than 85% agreement for each of the five diagnoses tested, with Cohen's kappa ranging from 0.64 to 0.69. This substantial interrater agreement was confirmed with McNemar's test for marginal proportions ($p>0.99$ for all diagnoses).

A history of parental emotional maltreatment was assessed with the antipathy and neglect subscales of the Childhood Experience of Care and Abuse questionnaire (CECA.Q),[43] which examines emotional maltreatment by parents from birth to age 17. All items from the questionnaire were derived from the well-validated CECA interview.[44] On a 5-point Likert type scale ranging from (Yes, Definitely) to (No, Not at All), the antipathy (mother and father) scale assessed hostile and rejecting parenting (e.g., "She was very critical of me," "He made me feel unwanted"), and the neglect subscale assessed the extent to which parents

provided material or emotional support for their children (e.g., “he was concerned about my whereabouts,” and “she tried to make me feel better when I was upset”). A one unit increase/decrease in maltreatment reflects a unit increase on the 1 to 5 scale. The neglect scale was highly correlated with the antipathy subscale for both parents (Mothers: r=0.72, p<0.0001; Fathers: r=0.77, p<0.0001). In accord with prior work we combined both scales;[45] maternal antipathy and neglect and paternal antipathy and neglect scores were averaged to create an overall *mother emotional maltreatment* composite, and *father emotional maltreatment* composite.

The CECA.Q possesses good test-retest reliability and alternate forms of reliability when compared to the CECA interview in both community and clinical populations.[46] Furthermore, the antipathy and neglect scales of the CECA.Q converges with other popular measures of childhood adversity, including the Parental Bonding Instrument,[47] suggesting this measure possesses good convergent validity. The questionnaire instructs respondents to fill out the CECA.Q in reference to the mother and father figure who they were “with the longest,” or the one they found “most difficult to live with.” Ninety-four percent of participants filled out the questionnaire in reference to their biological mother, and 93% responded in reference to their biological father.

The Center for Epidemiological Studies Depressive Symptoms Scale (CES-D) provided data on current depressive symptoms.[48, 49] Studies have shown acceptable test-retest reliability and excellent construct validity.[49] It has been widely used in cancer studies.[50]

The Pittsburgh Sleep Quality Index provided data on sleep quality and sleep disturbances.[51]

The Older Adults Resources Survey (OARS) Multidimensional Functional Assessment Questionnaire assessed underlying diseases, as well as associated medications.[67] Several studies have found excellent agreement between self-reports and hospital or physician records for specific conditions including myocardial infarction, stroke, and diabetes.[52, 53]

Statistical Methods

The mRNA markers (levels of CD25, CD3ε, ICAM-1, and CD68) were highly correlated, with pairwise Spearman’s correlation coefficients ranging from 0.78 to 0.91 and a Cronbach’s alpha of 0.95 (using log-transformed values). Thus, a single composite mRNA index was created for each subject using z-scores. For each subject, the z-scores for each (base 10) log-transformed mRNA marker were calculated, and these four z-scores were averaged to produce the summary construct. These cell surface markers operate together in vivo; this combined index reflects this coordinated immune response to the BCC tumor. We used this average mRNA z-score as the primary outcome of interest. Secondary analyses used depressive symptoms (CES-D) scores as the outcome.

Linear regression analyses were conducted to evaluate relationships between parental emotional maltreatment, the experience of severe life events, and each outcome. The LEDS life events variable was dichotomized at none versus one or more in the past year. Adjusted models controlled for age and gender. Models for the mRNA z-score index additionally controlled for smoking status, alcohol consumption, comorbid conditions, sleep quality (Pittsburgh Sleep Quality index), and BCC tumor type. Residual plots were examined for all models, and when the normality assumption was found to have failed, outcomes were log-transformed. Alpha was set to 0.05, and two-sided tests were conducted. All analyses were performed in SAS version 9.1.

Results

Participant sample characteristics are summarized in Table 1. There were 48 males and 43 females in the sample, the majority of whom had at least some college education (n=72, 79%). This is a cancer that occurs among those with fair skin, and all participants self described themselves as white.[54] The average age at interview was 58.2 years (SD=13.5). The 91 participants were generally healthy with few comorbid conditions: n=4 (4%), asthma, n=5 (5%), emphysema, n=2 (2%), heart disease, n=8 (9%), hypertension, n=27 (30%), kidney disease, n=2 (2%), liver disease, n=1 (1%), stroke, n=2 (2%) and thyroid disease, n=4 (4%). Comorbid conditions did not include psychiatric conditions. Neither father nor mother's emotional maltreatment was associated with age (Mothers: r=.14, p=0.20, Fathers: r=0.17, p=0.11). Parental maltreatment was not associated with the experience of any severe life events (Mothers: r=0.10, p=0.35, Fathers: r=0.17, p=0.10).

Table 2 summarizes the association of mother's emotional maltreatment and life events with the average mRNA z-score. In the unadjusted model there was a significant interaction between mother's emotional maltreatment and the experience of any severe life events, and this interaction persisted in the adjusted model (unadjusted p=0.02, adjusted p=0.009). The experience of severe life events led to a negative association between mother's emotional maltreatment and mRNA z-score. In the adjusted model, a one unit increase in mother's emotional maltreatment was not significantly associated with mRNA z-score for participants with no life events (B=0.020, 95% CI: −0.024 to 0.065, p=0.37). However, for participants with one or more severe life events, a one unit increase in maternal emotional maltreatment score led to a 0.086 point decrease in mRNA z-score (95% CI: −0.15 to −0.017, p=0.02). This interaction is illustrated in Figure 1.

Table 3 summarizes a similar interaction between father's emotional maltreatment and life events in predicting mRNA z-score (unadjusted p=0.02, adjusted p=0.03). In the adjusted model, a one unit increase in father's emotional maltreatment was significantly associated with a 0.063 point decrease in mRNA z-score (95% CI: −0.12 to −0.010, p=0.02) for participants who had experienced any severe life events. Participants who had not experienced any life events did not have a significant relationship between father's emotional maltreatment and mRNA z-score (B=0.009, 95% CI: −0.031 to 0.050, p=0.65).

Additional analyses evaluated the effect of removing father's emotional maltreatment from the adjusted models in Table 2 and mother's emotional maltreatment from the adjusted model in Table 3 because father's and mother's emotional maltreatment were moderately correlated (r=0.42, p<0.0001). Removing paternal emotional maltreatment had negligible effects on estimates for maternal emotional maltreatment and vice versa in both mRNA z-score models. We also considered an interaction between mother's and father's emotional maltreatment to assess any added effect when both parents emotionally maltreated their children; results were non-significant.

Post-hoc analyses were performed by repeating the models in Tables 2 and 3 using each of the individual mRNA markers (log-transformed) as the outcome (8 total separate adjusted regression models). Results matched the results of the composite models and were consistent across all mRNA variables. The emotional maltreatment by LEDS interaction significantly predicted each individual mRNA variable in all but one model; the only non-significant interaction was in the model using paternal emotional maltreatment predicting CD3ε, and the effect was in the expected direction (p=0.13).

A separate linear regression model to assess the relationship between parental emotional maltreatment, severe life events, and CES-D is presented in Table 4. The interaction between parental emotional maltreatment and life events predicting depressive symptoms

was not significant, so we dropped the interaction term from Table 4 as is the standard convention.[55] Father's emotional maltreatment was positively associated with depressive symptoms. A one unit increase in father's emotional maltreatment was associated with a 4.0% increase in the CES-D score (p=0.03). Neither mother's emotional maltreatment nor severe life events were significantly associated with CES-D, though slope estimates were in the expected direction. A history of major depression (as assessed by the SCID) was not associated with mother's emotional maltreatment (Wilcoxon rank sum p=0.80), father's emotional maltreatment (Wilcoxon rank sum p=0.08), or severe life events (chi-square p=0.27).

The CES-D scores were not associated with mRNA z-score in unadjusted or adjusted models and did not mediate the effects of parental emotional maltreatment on mRNA z-score (results not shown). The same conclusions held when we looked at the effect of a history of major depression (as assessed by the SCID) in place of CES-D. Adding CES-D or history of major depression as a predictor did not change the point estimates or significance of father's or mother's emotional maltreatment or life events or their interaction in the models presented in Tables 2 and 3. In the CES-D model (Table 4), removing maternal emotional maltreatment resulted in a small (4%) increase in the point estimate for paternal emotional maltreatment, with a subsequent decrease in the p-value to p=0.01. Adding sunburn history, skin type, and/or time since life events as predictors did not change the point estimates or significance levels of our results presented in Tables 2 and 3. If we included illness related severe events, only two additional people fell into the severe life event group; all of the analyses remained the same (i.e. point estimates did not change substantially, and significance levels were identical). The number of previous BCCs did not predict differences in mRNA levels or alter our findings. Finally, the pattern of results remained the same when the antipathy and neglect subscales of the CECA.Q were assessed independently.

Discussion

Our results show that among BCC patients who experienced a severe stressor in the past year, those who were emotionally maltreated by their mothers or fathers as children were more likely to have poorer immune responses as reflected in lower levels of mRNA for CD25, CD3ε, ICAM-1, and CD68 to their BCC tumors. Being emotionally maltreated by one's father was also linked to higher depressive symptoms. However, depressive symptoms and a history of depression, *per se*, were not directly linked to the BCC immune responses related to the BCC tumor. Females had a poorer immune response to the BCC tumor than males; most of the prior literature on BCC immune responses has not analyzed responses by sex, so it is unclear if this is typical or not.

The immune system plays a prominent role in response to BCC tumors because they are immunogenic, unlike many other common cancers that do not show the same responsiveness to the immune system.[23] Studies addressing control of BCC tumor progression show that inflammatory cells both in the peritumoral milieu and those that infiltrate BCC tumors have important roles.[24, 56, 57] Although key risk factors for a person's first BCC include childhood sun exposure, fair skin, and being male, subsequent tumors are not reliably related to these variables.[21, 22] Psychological stress may play an important role in the tumor environment for this immunogenic tumor, and have important implications for subsequent BCC tumors. Future studies should further investigate the clinical implication of the current findings.

Mechanistically, troubled parent-child relationships can alter the set point for the stress response system. Individuals who had adverse childhood relationships are more

physiologically reactive to stress as adults compared to those that did not.[58] As described previously, early life adversity has been linked to subsequent dysregulated immune function in adults, and physiological responsiveness to subsequent stressors.[11–14, 59]

We examined mRNAs encoding for proteins that are expressed on various immune cells and have been implicated in their function; mRNAs carry the information that specify the properties of the protein end product. The expression of mRNAs for CD3ε, CD68, CD25, and ICAM-1 indicates a coordinated immune response to the BCC tumor. In general, BCC tumor tissue has higher levels of mRNA immune markers than normal tissue because the immune system is responding to the tumor. The presence of these markers in BCC tumors is suggestive of infiltration of immune cells as part of the anti-tumor immune response.[24, 56, 60–63]

This study extends animal work demonstrating that stress, especially early in life, can impact tumor growth and progression.[17] Our findings complement work demonstrating that early life stress increases vulnerability to tumor development when exposed to an additional stressor in adulthood.[17]

Our findings also complement studies from Lutgendorf and colleagues that have addressed the relationship between psychosocial factors and immune markers within the tumor environment.[63, 64] Ovarian cancer patients who were more distressed had poorer natural killer (NK) cell activity in tumor-infiltrating lymphocytes than those who were less distressed.[64, 65] Furthermore, those who had more social support had greater NK cell activity in tumor-infiltrating lymphocytes than those who had less support. The current study is the first to show that early life stressors can also influence the tumor environment in humans.

Our work may have broader implications for other cancers. In a large prospective study with over one million participants, those with nonmalignant skin cancers were 20–30% more likely to die from other noncutaneous cancers; the relative risk for mortality from other cancers was 1.30 in men and 1.26 in women.[66] A recent meta-analysis reported that individuals who were more stress reactive were at greater risk for cancer mortality those who were not.[67] Accordingly, BCCs may have some prognostic value for broader cancer risks.

These findings could also be relevant to recent work linking child maltreatment with cancer incidence. One study demonstrated a dose-response relationship between the number of exposures to abuse or household dysfunction during childhood and cancer incidence.[16] In other work, those who were physically abused as children had 49% higher odds of having a cancer diagnosis than those who were not abused.[68] The findings remained after adjusting for health behaviors such as smoking and exercise; immune dysregulation may have contributed to this link.[68]

One major strength of this study was the use of the LEDS to assess life events. The LEDS allowed us to exclude life events that were related to underlying medical issues. Furthermore, because the LEDS uses objective ratings of stress severity, biases related to depressive symptoms do not influence people's ratings of their life stress. Accordingly, the LEDS allowed us to assess links between severe stressors and immune function with control over biased reporting of stress related to depressive symptoms and poor health.

Despite the potential importance of understanding how early adverse experiences influence cancer risk, there are limitations that should be acknowledged. Our participants could have been biased when reporting the degree to which their parents emotionally maltreated them as children. However, adults generally underreport rather than over report childhood abuse and

neglect.[69] In addition, there are many other forms of child adversity not assessed in the current study that could also impact immune function such as low socioeconomic status.[70] Future work should take these factors into account as well. Another limitation is our exclusive focus on BCC tumors. We chose this disease because of the known immunogenic properties of BCC tumors, but future work assessing other types of tumors will be important in order to generalize our findings to cancer more broadly. Finally, our sample was exclusively white, which is not surprising given the nature of the disease.

BCC is a substantial public health concern; it is highly prevalent and carries risks for scarring and disfigurement.[21] Furthermore, it may be prognostic for other cancers. A better understanding of the factors that contribute to BCC incidence and reoccurrence is clinically relevant. Troubled early parental experiences are linked to greater stress reactivity in adulthood, and poorer immune regulation. This is the first study to show troubled early parental experiences, in combination with a severe life event in the past year, predict local immune responses to a BCC tumor. These data complement and expand growing evidence that the consequences of early parental experiences extend well beyond childhood.

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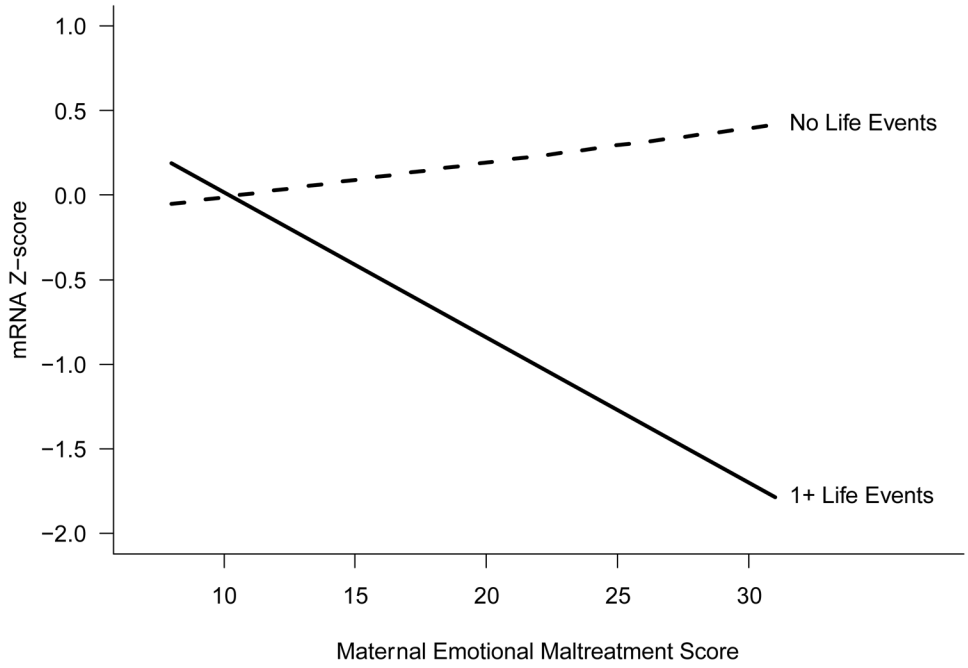

Figure 1.
Interaction plot illustrating the relationship between mother's emotional maltreatment, severe life events, and composite mRNA z-score, from adjusted model in Table 2. Values of all other variables in the model are set to their sample means.

Table 1

Characteristics of the Study Sample (n=91)

Demographics	-	-
Female sex, Number (%)	43	(47)
White race, Number (%)	91	(100)
Education, Number (%)		
High school or less	19	(21)
Some college	26	(29)
College degree	46	(51)
Marital status, Number (%)		
Single	7	(8)
Married	69	(76)
Common law	2	(2)
Divorced	9	(10)
Widowed	4	(4)
Age (years)		
Mean (SD)	58.2	(13.5)
Range	23–92	
Self-Report Measures		
Number of LEDS events, Number (%)		
0	70	(77)
1	17	(19)
2+	4	(4)
Months from most recent LEDS event to biopsy (n=21)		
Mean (SD)	5.5	(3.2)
Range	0.1–11	
History of major depression, Number (%)	30	(33)
Center for Epidemiological Studies Depression Scale (CES-D)		
Mean (SD)	7.8	(9.0)
Median (IQR)	6	(2–9)
Range	0–49	
Pittsburgh Sleep Quality Index (PSQ)		
Mean (SD)	4.9	(2.7)
Range	0–15	
Any comorbid conditions, Number (%)	65	(71)
Current smoker, Number (%)	10	(11)
Consume alcohol, Number (%)	46	(51)
Emotional Maltreatment (CECA.Q)		
Mother		
Mean (SD)	12.6	(5.5)
Range	8–31.5	
Father		

Mean (SD)	14.4	(6.5)
Range	8–31	
mRNA Levels		
CD25		
Median (IQR)	38	(12–103)
Range	2.5–1284	
CD3ε		
Median (IQR)	0.23	(0.10–0.56)
Range	0.020–16	
ICAM-1		
Median (IQR)	0.24	(0.13–0.50)
Range	0.015–3.5	
CD68		
Median (IQR)	1.5	(0.56–3.6)
Range	0.11–38	
Clinical Characteristics		
BCC tumor type, Number (%)		
Nodular	27	(30)
Superficial	15	(16)
Mixed type	49	(54)
BCC site, Number (%)		
Head and neck	50	(55)
Trunk	17	(19)
Upper limbs	15	(16)
Lower limbs	3	(3)
Multi-site	6	(7)
Skin type - Burning, Number (%)		
Always burn	11	(12)
Usually burn	17	(19)
Burn moderately	31	(34)
Burn minimally	21	(23)
Rarely/never burn	11	(12)
Number of sunburns before age 19, Number (%)		
0	4	(4)
1–9	44	(48)
10–19	23	(25)
20+	19	(21)
Unknown	1	(1)

Table 2

Association of mother's emotional maltreatment and LEDS events with average mRNA z-score. Results from linear regression models (n=91).

	Unadjusted Model $R^2 = 0.08$			Adjusted Model $R^2 = 0.28$		
Variable	**Estimate**	**(95% CI)**	**p-value**	**Estimate**	**(95% CI)**	**p-value**
Mom's emotional maltreatment	0.0077	(−0.033, 0.048)	0.71	0.020	(−0.024, 0.065)	0.37
Any LEDS Events	0.98	(−0.099, 2.1)	0.07	1.1	(0.044, 2.1)	**0.04**
Mom's emotional maltreatment x Any LEDS Events	−0.10	(−0.18, −0.019)	**0.02**	−0.11	(−0.18, −0.028)	**0.009**
Dad's emotional maltreatment				−0.013	(−0.046, 0.021)	0.45
Age (years)				−0.0019	(−0.017, 0.013)	0.81
Female (ref: male)				−0.45	(−0.86, −0.038)	**0.03**
Comorbid conditions				−0.060	(−0.28, 0.16)	0.59
Smoker (ref: non-smoker)				−0.037	(−0.63, 0.56)	0.90
Drinks alcohol (ref: non-drinker)				0.037	(−0.35, 0.42)	0.85
Pittsburgh Sleep Quality Index				0.034	(−0.036, 0.11)	0.34
Tumor type (ref: Mixed)						
Nodular				−0.81	(−1.2, −0.40)	**0.0002**
Superficial				−0.38	(−0.90, 0.14)	0.15

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Table 3

Association of father's emotional maltreatment and LEDS events with average mRNA z-score. Results from linear regression models (n=91).

	Unadjusted Model $R^2 = 0.08$			Adjusted Model $R^2 = 0.26$		
Variable	**Estimate**	**(95% CI)**	**p-value**	**Estimate**	**(95% CI)**	**p-value**
Dad's emotional maltreatment	0.010	(−0.027, 0.048)	0.59	0.0094	(−0.031, 0.050)	0.65
Any LEDS Events	1.1	(−0.073, 2.2)	0.07	1.0	(−0.14, 2.2)	0.08
Dad's emotional maltreatment x Any LEDS Events	−0.076	(−0.14, −0.011)	**0.02**	−0.073	(−0.14, −0.0086)	**0.03**
Mom's emotional maltreatment				−0.010	(−0.049, 0.030)	0.63
Age (years)				−0.0025	(−0.018, 0.013)	0.75
Female (ref: male)				−0.39	(−0.80, 0.023)	0.06
Comorbid conditions				−0.0090	(−0.23, 0.21)	0.94
Smoker (ref: non-smoker)				−0.090	(−0.70, 0.52)	0.77
Drinks alcohol (ref: non-drinker)				−0.076	(−0.46, 0.31)	0.69
Pittsburgh Sleep Quality Index				0.023	(−0.049, 0.10)	0.52
Tumor type (ref: Mixed)						
Nodular				−0.80	(−1.2, −0.38)	**0.0003**
Superficial				−0.42	(−0.94, 0.11)	0.12

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Table 4

Association of maternal and paternal emotional maltreatment and LEDS events with depressive symptoms (CES-D*). Results from a linear regression model (n=91).

	Adjusted Model $R^2 = 0.15$		
Variable	**Estimate**	**(95% CI)**	**p-value**
Age (years)	−0.005	(−0.020, 0.010)	0.51
Female (ref: male)	0.25	(−0.16, 0.66)	0.24
Any LEDS Events	0.32	(−0.16, 0.79)	0.19
Mom's emotional maltreatment	0.004	(−0.037, 0.044)	0.85
Dad's emotional maltreatment	0.039	(0.0043, 0.074)	**0.03**

*Outcome (CES-D) is natural log transformed