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Sickness behavior induced by cisplatin chemotherapy and radiotherapy in a murine head and neck cancer model is associated with altered mitochondrial gene expression

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Abstract

The present study was undertaken to explore the possible mechanisms of the behavioral alterations that develop in response to cancer and to cancer therapy. For this purpose we used a syngeneic heterotopic mouse model of human papilloma virus (HPV)-related head and neck cancer in which cancer therapy is curative. Mice implanted or not with HPV+ tumor cells were exposed to sham treatment or a regimen of cisplatin and radiotherapy (chemoradiation). Sickness was measured by body weight loss and reduced food intake. Motivation was measured by burrowing, a highly prevalent species specific behavior. Tumor-bearing mice showed a gradual decrease in burrowing over time and increased brain and liver inflammatory cytokine mRNA expression by 28 days post tumor implantation. Chemoradiation administered to healthy mice resulted in a mild decrease in burrowing, body weight, and food intake. Chemoradiation in tumor-bearing mice decreased tumor growth and abrogated liver and brain inflammation, but failed to attenuate burrowing deficits. PCR array analysis of selected hypoxia and mitochondrial genes revealed that both the tumor and chemoradiation altered the expression of genes involved in mitochondrial energy metabolism within the liver and brain and increased expression of genes related to HIF-1α signaling within the brain. The most prominent changes in brain mitochondrial genes were noted in tumor-bearing mice treated with chemoradiation. These findings indicate that targeting mitochondrial

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dysfunction following cancer and cancer therapy may be a strategy for prevention of cancer-related symptoms.

Keywords

Cancer; Human papilloma virus; Sickness behavior; Chemoradiotherapy; Hypoxia; Mitochondria

1. Introduction

Cancer and its therapy are associated with symptoms including fatigue and depressed mood. These symptoms are often present at diagnosis, peak during therapy, and can persist long after completion of therapy. As these symptoms have a striking parallel to inflammation-induced sickness behavior [1, 2], the inflammation hypothesis has been the primary mechanism under investigation. Preclinical models of cancer confirm that tumors induce inflammation in the periphery and brain [3-5] and a few reports indicate that chemotherapy agents can increase brain expression of proinflammatory cytokines [6-8]. Moreover, various clinical studies report associations between cancer-related symptoms and various biomarkers of inflammation [9-13]. However, there are also numerous reports where no association between symptoms and cytokines could be identified [14-18]. This is not surprising as chemotherapy is often immunosuppressive [19, 20]. Therefore, other mechanisms, including mitochondrial dysfunction, have been proposed as potential mechanisms of chemotherapy-induced symptoms [21]. The objective of the present study was to investigate the mechanisms of the behavioral alterations that develop in response to cancer and cancer therapy in an animal model of cancer. We hypothesized tumor-induced behavioral changes would be linked to inflammation, while chemoradiation-induced behavioral changes would be more closely associated with alterations in mitochondrial gene expression. For this purpose we selected an inflammatory and metabolically active murine oropharynx squamous cell carcinoma model in which mice undergo heterotopic implantation of tonsil epithelial cells transfected with H-Ras and human papilloma virus (HPV) oncogenes E6 and E7 [22]. The rates of HPV-related head and neck cancer are on the rise, particularlly among middle aged white males [23, 24]. Although HPV-related head and neck cancer responds relatively well to a regimen of chemotherapy and radiotherapy (chemoradiation) [25], this treatment is associated with the development of important local and systemic symptoms including mucositis, pain, fatigue, and distress [26-28].

The tumor developed by mice injeced into their hind leg with HPV-related tumor cells responds to a combined regimen of cisplatin chemotherapy and radiotherapy similar to the one used in HPV-related head and neck cancer [22]. Mice implanted or not with tumor cells were exposed or not to chemoradiation according to a 2 × 2 factorial design. Sickness was assessed by decreases in body weight and food intake and reduced burrowing, a species-specific motivated behavior that is very sensitive to variations in well being [29, 30]. Using this model we confirmed that the signs of sickness that developed in tumor bearing mice were associated with inflammation propagating from the tumor to the liver and brain. However, the signs of sickness that developed in tumor bearing mice treated with chemoradiation were no longer associated with inflammation. In view of the highly

metabolic nature of the tumor [31, 32] and the well known damaging effects of cisplatin on mitochondria [33-39], we investigated the relationship between behavioral alterations and expression of genes involved in mitochondrial energy metabolism and hypoxia in the liver and brain using PCR arrays. We observed additive effects of tumor and chemoradiation on burrowing and alterations in expression of genes involved in mitochondrial energy metabolism in the brain, pointing to mitochondrial dysfunction as a possible cause of cancer-related symptoms.

2. Materials and Methods

2.1 Mice

All procedures described in this study were approved by the Institutional Animal Care and Use Committees of the University of Texas MD Anderson Cancer Center. Experiments were conducted on adult male C57BL/6 mice individually housed in temperature and humidity controlled environments on 12 hour light-dark cycles. Food and water were available *ad libitum*.

2.2 Tumor Model

A heterotopic syngeneic murine tumor model of HPV-related head and neck cancer was used. This model has been described in detail previously [22, 40, 41]. The tumor cells were derived from normal C57BL/6 mouse oropharyngeal epithelial cells that were transfected with HPV E6 and E7 oncogenes and hRAS. Mice were inoculated with 1×10^6 tumor cells into the right hind leg. The advantage of this heterotopic location is that tumor growth does not interfere with eating and drink, as would be observed if implanted in the oral cavity, and it allows for irradiation to be presented to the tumor without direct effects on the brain or abdominal cavity. Tumor volume was determined from three mutually orthogonal tumor diameters (d1, d2, d3) measured using Vernier calipers [Volume = $(\pi/6)(d1*d2*d3)$] as previously described [42, 43].

2.3 Cancer therapy

Mice were treated with a regimen of cisplatin chemotherapy and radiotherapy that reliably suppresses tumor growth [41]. This regimen included 3 rounds of once weekly cisplatin (Calbiochem, EMD Millipore, Billerica, MA) plus 8 Gy local tumor irradiation of the leg beginning 12 days after tumor implantation. Cisplatin was dissolved in sterile saline and administered by intraperitoneal injection at a dose of 5.28 mg/kg (equivalent to approximately 20 mg/m^2). Radiotherapy was administered via a small animal $cesium^{137}$ irradiator that collimates the parallel opposed radiation beams to a 3 cm diameter circular field and thereby avoids irradiation of surrounding tissues, including the abdomen.

2.4 Assessing Sickness

Sickness was assessed by evaluating body weight, food consumption (by measuring food disappearance), and burrowing. For the burrowing task, mice were provided access to a slightly elevated tube filled with 200 +/− 1.0 g of standard rodent chow. The amount of chow removed from the tube after 30 min was recorded [29, 30]. Healthy mice exposed to the burrowing task usually remove most of the food pellets from the burrowing tube during

the 30 min time period they are allocated and generally do not eat them. Mice were trained for 3-5 sessions prior to baseline assessment. Mice burrowing less than 30 grams following training were excluded from the experiment as non-burrowers (< 5% of mice).

2.5 Tissue Collection

Mice were euthanized by CO_2 inhalation. For assessment of gene expression, mice were saline perfused and tissue (i.e., brain, liver, and tumor tissue) was collected, snap-frozen in liquid nitrogen, and stored at −80°C until RNA extraction. Brain tissue was crushed with a mortar and pestle on liquid nitrogen and divided for assessment of inflammatory cytokines and for assessment of mitochondrial energy metabolism and hypoxia signaling by RT^2 Profiler PCR arrays. To verify the lack of metastatic disease in the liver, liver tissue was fixed with 4% formalin and stained with hematoxylin and eosin (H&E).

2.6 Real-time PCR for inflammatory cytokines

For assessment of inflammatory cytokines, total RNA was isolated using TRIzol reagent (Ambion by Life Technologies, Grand Island, NY) and E.Z.N.A. RNA Isolation Columns (Omega Bio-Tek, Norcross, GA). Reverse transcription was conducted using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems by Life Technologies, Grand Island, NY) according to manufacturer's instructions.

Real time RT-PCR was carried out with TaqMan gene expression assays. Targets include IL-6 (cat number: Mm.PT.51.12387735), IL-1β (cat number: Mm.PT.51.17212823), TNF-α (cat number: Mm.PT.51.16622079) and GAPDH (cat number: Mm.PT.39a.1) from Integrated DNA Technologies (Coralville, IA). GAPDH served as the endogenous housekeeping control. Reactions were performed in duplicate, and relative fold difference for each target gene was calculated using the $2^{-\Delta\Delta CT}$ method, where CT is the threshold concentration.

2.7 RT^2 Profiler PCR arrays of Hypoxia Signaling Pathways and Mitochondrial Energy Metabolism

Total RNA was isolated from brain and liver murine samples using TRIzol reagent (Ambion by Life Technologies, Grand Island, NY) and E.Z.N.A. RNA Isolation Columns (Omega Bio-Tek, Norcross, GA). Approximately 120 ng of extracted RNA were converted to first strand cDNA using the RT^2 First Strand Kit (SA Biosciences, Qiagen, Valencia, CA) according to the manufacturer's instructions. The mouse Mitochondrial Energy Metabolism RT^2 Profiler PCR Array (PAMM_008ZE-4, SA Biosciences, Qiagen, Valencia, CA) and the mouse Hypoxia Signaling Pathway RT^2 Profiler PCR Array (PAMM-032Ze-4, SA Biosciences, Qiagen, Valencia, CA) were run according to manufacturer's instructions. The Mitochondrial Energy Metabolism array assesses the expression of 84 genes involved in modulating ATP synthesis and oxidative phosphorylation. The Hypoxia Signaling Pathway array evaluates 84 genes that are responsive to low oxygen levels. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and β-actin (*ACTB*) were used as reference genes. All samples were tested in duplicate. Reactions were carried out on ABI PRISM 7900HT sequence detection system.

The data were normalized and analyzed using fold change from control with positive values indicating an upregulation and negative values indicative of a downregulation compared to control. Canonical pathway analyses were conducted using Ingenuity Pathway Analysis (Qiagen, Silicon Valley, Redwood City, CA). In this analysis, all groups were independently compared to the control mice (no tumor, no chemoradiation). Heat maps were generated from the log transformed fold change using CIMminer (http://discover.nci.nih.gov/cimminer).

2.8 Hematoxylin and Eosin (H&E) staining for liver pathology

Fixed liver sections were evaluated by a pathologist from the Department of Veterinary Medicine and Surgery from MD Anderson Cancer Center. The pathologist also scored hypertrophy of Kupffer cells on a 4 point scale (Grade 1= modest, rare < 10%, Grade 2 = mild, infrequent 10-20%, Grade 3 = moderate, frequent 20-50%, and Grade 4 = severe, extensive > 50%).

2.9 Experimental Design

To evaluate the effects of chemoradiation in tumor-bearing animals, we employed a 2 (control vs tumor) × 2 (placebo vs chemoradiation) factorial design (n=7 mice/group). Mice were injected or not with tumor cells on day 0 and treated or not with chemoradiation on days 12, 19, and 26. Brain, liver, and tumor were collected one day after the final session of chemoradiation for analysis. Signs of sickness were measured prior to tumor implantation (baseline), prior to the start of chemotherapy (day 6), and the day after each session of chemoradiation (day 13, 20, and 27). In a separate group of animals (n=4 mice/group), liver tissue was collected to evaluate for possible tumor metastasis. On day 28 post-tumor implantation tissue was collected from healthy and tumor-bearing mice and was stained for H&E.

2.10 Statistical Analysis

Data were analyzed using SPSS (version 19, Chicago, IL). Data are expressed as mean +/− standard error of the mean. Depending on the experimental design, one- or two-way analyses of variances (ANOVAs) with tumor and chemoradiation as between subject factors were run to evaluate differences in cytokine expression levels. Two-way repeated measures ANOVAs were conducted to evaluate behavioral changes over time. *Post hoc* analyses were conducted with the least significant test to clarify group differences, as needed. *P*-values of less than 0.05 were considered significant.

3. Results

3.1. Chemoradiation and HPV-related tumors induce sickness

In line with previously reported data using this murine model of HPV-related head and neck cancer (Spanos et al., 2009), tumor growth was inhibited by chemoradiation ($F(3,36) = 13.76$, $p<0.001$; Figure 1A). Chemoradiation significantly reduced body weight over time in control and tumor-bearing mice, $F(4,96)=16.10$, $p<0.001$ (Figure 1B), and reduced food consumption in the 24 h following each treatment, $F(7,168)=5.35$, $p<0.001$ (Figure 1C). There was no evidence of cachexia or anorexia in tumor-bearing mice. Instead, a subtle

increase in food consumption was observed in tumor-bearing mice toward the end of the experiment, $F(7,168)=2.13$, $p<0.05$.

Burrowing levels declined significantly over time in tumor bearing mice, $F(4,96)=8.07$, $p<0.001$, with a greater than 40% decline in performance by day 27. Further, chemoradiation significantly suppressed burrowing in control mice and further suppressed burrowing in tumor-bearing mice, $F(4,96)=2.95$, $p<0.05$ (Figure 1D). Tumor-bearing mice treated with chemoradiation showed reduced burrowing following the first session of chemoradiation, while sham-treated tumor-bearing mice showed a more gradual decline in performance.

3.2 Chemoradiation abrogates tumor-induced liver and brain proinflammatory cytokine expression

Analysis of the tumor showed no significant change in proinflammatory cytokine expression following 3 rounds of cisplatin and tumor irradiation (Figure 2A). There was a trend toward a decrease in tumor IL-1β mRNA expression within the tumor following chemoradiation ($p<0.1$). However, in view of the robust decrease in tumor volume following chemoradiation, the total cytokines produced by the tumor would likely be much lower.

Tumor-bearing mice had increased proinflammatory cytokine expression in the liver (Figure 2B) and brain (Figure 2C). Within the liver IL-6, IL-1β, and TNF-α mRNA expression (all $p<0.01$) were elevated and within the brain IL-1β was elevated ($p<0.05$). Chemoradiation significantly abrogated these effects (all $p<0.05$). Despite the fact that tumor-bearing mice treated with chemoradiation had cytokine expression levels that were not different from healthy controls, they showed the most severe behavioral effect.

3.3 Inflammation is associated with tumor volume in tumor bearing mice

In view of the natural variability in tumor volume in tumor bearing mice we searched for possible associations between tumor size and cytokine expression within the liver and brain. As anticipated, there was a significant positive correlation between tumor volume and liver IL-6, IL-1β, and TNF-α as well as brain IL-1β mRNA expression (Table 1). This provides confirmation that the suppression in cytokine expression in liver and brain observed following chemoradiation is related to reducing the tumor volume.

3.4 Both the tumor and chemoradiation are associated with altered expression of genes related to mitochondrial energy metabolism and hypoxia

To explore the possible role of mitochondial energy metabolims and hypoxia in cancer-related symptoms we conducted real time RT-PCR array analyses in the liver and brain collected 28 days after the start of the experiment. Within the liver, the presence of the tumor was associated with significant downregulation of mitochondrial respiratory chain complex genes (Figure 3, Table S1A). Chemoradiation attenuated tumor-induced changes in mitochondrial respiratory chain complex gene expression (see Table 2). Pathway analyses also indicated that the HIF-1α signaling pathway was upregulated within the liver in response to the tumor alone, while chemoradiation in the presence of the tumor reduced this upregulation (refer to Figure S1, Table S2A).

Within the brain a different pattern was observed. While both chemoradiation and the tumor resulted in changes in gene expression within the mitochondrial respiratory chain (Figure 4, Table S1B), the highest degree of change was observed in tumor-bearing mice treated with chemoradiation, particularly within complex II, III, and IV (Table 2). Further, pathway analyses indicates that both the tumor and chemoradiation resulted in the upregulation of genes associated with HIF-1α activation within the brain (Figure 2S, Table S2B).

3.5 There is no evidence of metastatic disease within the liver of tumor-bearing mice

Given that the inflammatory and mitochondrial gene expression changes observed within the liver showed an expression pattern that would be expected within a tumor microenvironment, we sought verify that these changes were not related to the development of metastatic disease. A pathologist evaluated H&E stained liver sections from a healthy and tumor bearing mice (n=4/group). No evidence of metastatic disease or hepatocyte hypertrophy was noted; however, the pathologist did note the presence of grade 2 (n=2) and 3 (n=2) hypertrophy of Kupffer cells in tumor-bearing mice. These activated cells are likely a significant source of the increased inflammation observed within the liver of tumor-bearing mice. All non-tumor bearing mice were assigned a score of 1 (see Figure S3 for representative images). A Pearson chi square analysis shows this difference to be statistically significant (χ^2 (2) = 8, p<0.05).

4. Discussion

The results from this study demonstrate that a syngeneic HPV-related tumor model induced sickness behavior and inflammation. In contrast, chemoradiation in tumor-bearing mice reduced tumor volume and inflammation within the liver and brain while exacerbating tumor-induced sickness behavior. Chemoradiation by itself induced sickness behavior but had no effect on cytokine gene expression in the liver and brain. Both the tumor and chemoradiation induced alterations in the expression of genes associated with mitochondrial energy metabolism within the liver and brain as well as activation of genes associated with HIF-α signaling within the brain.

4.1 Effects of the Tumor

Tumor-bearing mice developed deficits in burrowing without any evidence of anorexia and cachexia. Deficits in burrowing in these mice were associated with evidence of inflammation in the periphery and brain, as evidenced by increased IL-6, IL-1β, and TNF-α mRNA expression within the liver and increased IL-1β mRNA expression within the brain. As there is a strong role for inflammation in the pathogenesis of HPV-related head and neck cancers [44-46], it is not surprising to observe a robust inflammatory profile in tumor-bearing mice. Further, the finding of a sickness response in mice implanted with tumor cells is consistent with other studies that show behavioral and neuroinflammatory changes in animal models of cancer [3, 5].

The livers of tumor-bearing mice presented reduced expression of mitochondrial respiratory chain genes, which likely indicates a shift from mitochondrial energy metabolism toward glycolysis. Further support for this shift toward glycolysis can be observed within a cluster

of metabolism genes of the hypoxia array; specifically genes involved in glycolysis showed a general increase in expression within the liver of tumor-bearing mice (e.g., HK2, LDHA, and PFKP). Gene expression changes indicative of hypoxia signaling and alterations in mitochondrial respiratory chain complex genes were observed within the brain. In general, mild increases in genes encoding components of mitochondrial complex were observed (see Table 2). The functional implications of such an upregulation remain to be determined by e.g., assessing mitochondrial respiratory capacity. Similar changes have been described in the brain of aged mice [47] and the postmortem brains of unmedicated patients with psychiatric diseases [48]. These changes have been interpreted as evidence of mitochondrial dysfunction and oxidative damage; however, it is yet unknown whether they represents a compensatory increase or dysfunctional state.

Further, it is yet unclear whether brain expression of IL-1β or mitochondrial dysfunction is ultimately responsible for decreased burrowing in tumor bearing mice. Future studies will evaluate this through administration of an IL-1 receptor antagonist and/or mitochondrial protectant agents. It will also be necessary to examine whether the gene expression changes observed within the whole brain represent subtle widespread alterations or the diluted expression of strong localized changes within specific brain regions.

More research is needed to understand the mechanism by which the tumor alters the expression of mitochondrial genes in distant organs. It is possible that the effects observed in the liver are a downstream consequence of chronic inflammation. The gene expression pattern observed in the liver is in accordance with the metabolic signature of inflammation, characterized by a switch from oxidative phosphorylation to glycolysis [49]. The information concerning the impact of inflammation on brain mitochondrial activity is still quite limited. However, an altered pattern of mitochondrial respiratory chain complex genes has been reported in animal models of septic shock, with decreased mitochondrial complex I and possibly complex IV predominating [50, 51]. A possible explanation for the distant metabolic effects of tumors is that tumor-induced exosomes or microvesicles, which contain double-stranded DNA, RNA, and proteins (including HPV oncogene proteins E6 and E7) enter circulation and interact with liver and brain cells (e.g., innate immune cells) to induce distant metabolic changes [52, 53].

4.2 Effects of Chemoradiation

The chemoradiation protocol we used induced body weight loss, temporarily decreased food intake, and reduced burrowing. These findings are in line with the literature. Both cisplatin and radiation have been shown to reduce body weight [54-57]. Further, chemotherapy treatment can suppress voluntary activity, including decreased running wheel [17, 58-61] and open field activity [56, 57].

Although the behavioral alterations induced by cancer therapy are usually assumed to be mediated by inflammation [1, 6-8] there was no evidence of liver or brain proinflammatory cytokine gene expression in response to chemoradiation in mice implanted or not with tumor cells. The lack of an inflammatory response in mice exposed to chemoradiation in the present study also contrasts with the pronounced behavioral and neuroinflammatory effects that develops in response to partial body irradiation [62, 63]. However, these last effects are

likely secondary to gut leakage following irradiation of the abdominal cavity [64, 65], which did not occur in the hind leg irradiation model employed in the current study.

In contrast to the lack of effect of chemoradiation on inflammation there was clear evidence of altered mitochondrial energy metabolism and activation of hypoxia signaling pathway in response to chemoradiation. Cisplatin is already known to induce mitochondrial dysfunction through the formation of mitochondrial DNA adducts [37, 38]. Further, cisplatin-induced fragmentation of mitochondria has been described in kidneys of cisplatin-treated mice and can be prevented by pharmacological inhibition of dynamin-related protein-1 (Drp-1), a critical mitochondrial fission protein [33]. Radiation can enhance the effects of chemotherapy on mitochondrial dysfunction through generation of radical oxygen species [35].

As expected the curative regimen of chemoradiation used in this study decreased tumor volume in tumor-bearing mice [22]. It also abrogated the expression of peripheral and central proinflammatory cytokines, likely through suppression of tumor growth. Despite this inhibition of inflammation, chemoradiation did not reverse or attenuate sickness in tumor bearing mice. Rather tumor-bearing mice treated with chemoradiation showed severe sickness behavior. This indicates that while inflammation may mediate sickness associated with growth of this tumor, this is clearly not the case for signs of sickness associated with chemoradiation. While chemoradiation attenuated tumor-induced suppression of mitochondrial gene suppression in the liver, the highest level of mitochondria gene dysregulation was noted in the brains of tumor-bearing mice treated with chemoradiation. While some genes within complex I and III were down regulated within the brain, the majority were upregulated compared to healthy control mice. As previously mentioned the functional implication of this gene expression pattern is yet to be determined.

4.3 Conclusion

Our data on the relationship between inflammation and sickness in tumor-bearing mice indicates that inhibition of inflammation could provide a strategy for reducing disease-driven symptoms, as would be observed in advanced cancer patients. This strategy is likely to be therapeutically safe as inhibition of tumor-induced inflammatory cytokines can attenuate tumor growth [31, 66-68]. However, our data on the effects of chemoradiation in tumor bearing mice would indicate that inflammation is unlikely to be the cause of symptoms that develop in response to cancer, meaning the use of anti-inflammatory treatments is not justified in this case. Agents aimed at mitochondrial protection and biogenesis should be preferred if it is demonstrated that such agents reverse the behavioral alterations associated with cancer therapy without adversely impacting the response of the tumor to cancer therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- A murine model of cancer induces sickness behavior and inflammation.
- Chemoradiation attenuates tumor-induced inflammation.
- Chemoradiation does not attenuate tumor-induced sickness.
- Combined tumor and chemoradiation alters brain mitochondrial gene expression.

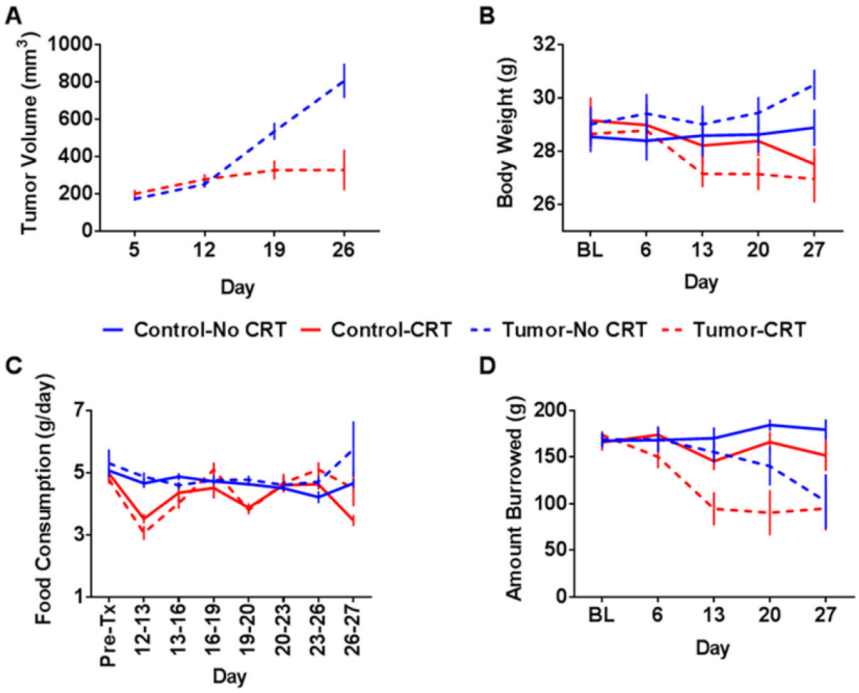

Figure 1. Sickness is induced by both chemoradiation and the tumor

Mice were implanted or not with tumor cells on day 0 and were exposed to chemoradiation (CRT) (5.28 mg/kg cisplatin + 8 Gy leg irradiation) or sham treatment on days 12, 19, and 26. (A) CRT suppressed tumor growth as indicated by a significant main effect of time ($p<0.001$), CRT ($p<0.05$), and the time by CRT interaction ($p<0.001$). (B) CRT also resulted in a reduction in body weight as indicated by a significant main effect of time ($p<0.05$) and a time by CRT interaction ($p<0.001$). (C) There was a significant main effect of time, CRT, and a time by CRT interaction (all $p<0.001$) for food consumption, indicating at transient reduction in consumption following CRT. (D) A main effect of time ($p<0.001$), CRT ($p<0.01$), tumor ($p<0.001$), a time by tumor interaction ($p<0.001$), and a time by CRT interaction ($p<0.05$) was observed for burrowing behavior. Data represented as mean +/− SEM. n=7 mice/group.

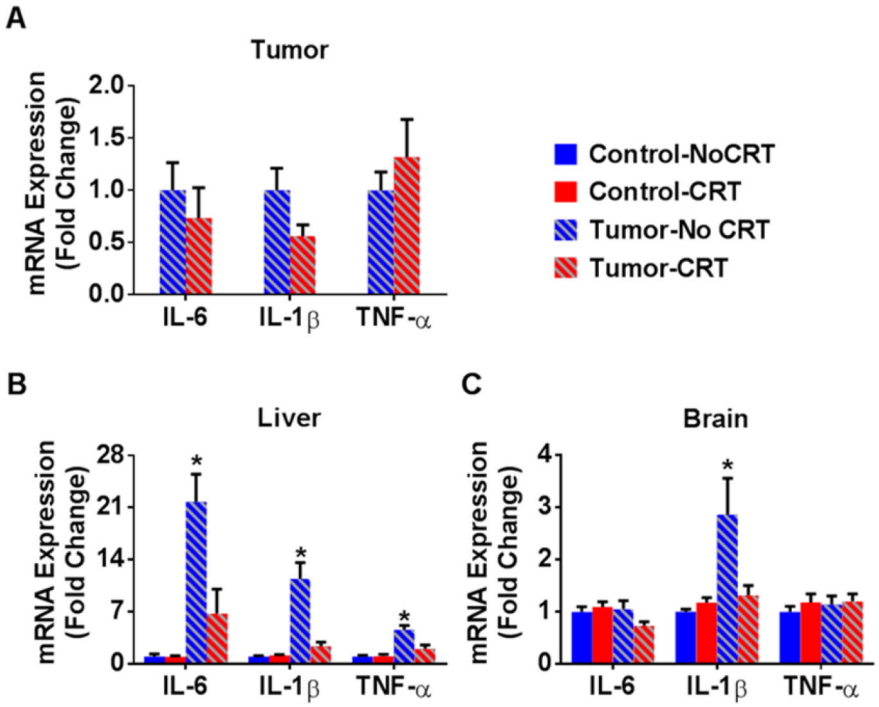

Figure 2. Tumor-induced cytokine expression is abrogated by chemoradiation

Mice were implanted or not with tumors on day 0 and were exposed to chemoradiation (5.28 mg/kg cisplatin + 8 Gy leg irradiation) or sham treatment on day 12, 19, and 26. Tissue was collected on day 27 and was analyzed by RT-PCR for proinflammatory cytokine expression. (A) There were no statistically significant effects of chemoradiation on tumor cytokine expression. (B) There were significant main effects of tumor and chemoradiation on liver cytokines (all *p*s<0.05) as well a significant tumor by chemoradiation interactions for IL-6, $F(1,24)=9.02$, $p<0.01$, IL-1β, $F(1,24)=16.89$, $p<0.001$, and TNF-α, $F(1,24)=9.89$, $p<0.005$. (C) Within the brain, there was a main effect of tumor, $F(1,24)=6.97$, $p<0.05$, and a tumor by chemoradiation interaction, $F(1,24)=4.48$, $p<0.05$, on IL-1β mRNA expression. *Post hoc* analyses show that untreated tumor-bearing mice had significantly higher cytokine mRNA expression. Data are represented as mean fold differences compared to control mice +/− SEM. * $p<0.05$, n= 7/group

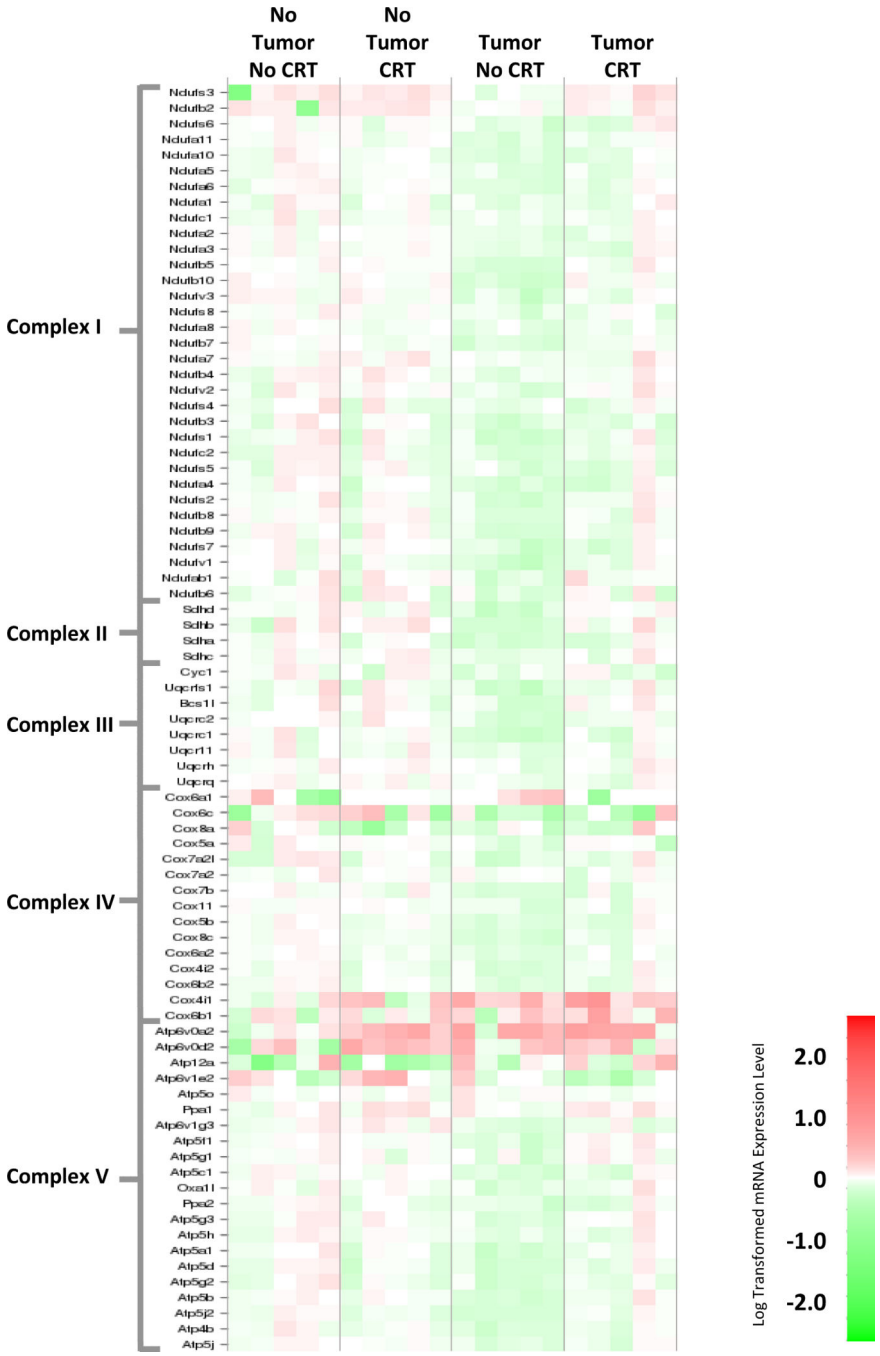

Figure 3. Heat map of mitochondria complex gene expression in the liver

Gene expression changes in mitochondrial complex genes in liver samples. Red corresponds to increased gene expression, while green corresponds to decreased expression.

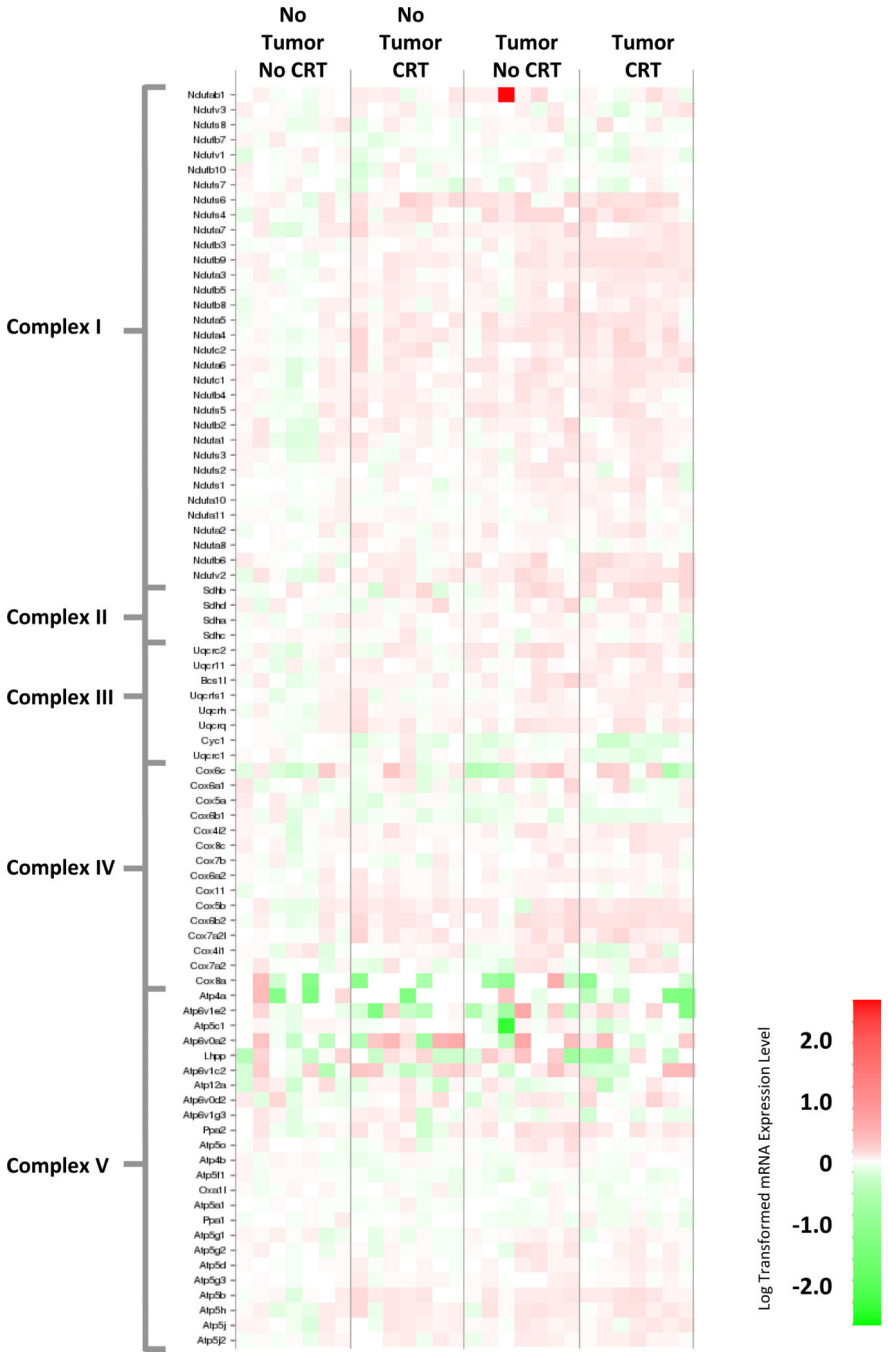

Figure 4. Heat map of mitochondria complex gene expression in the brain

Gene expression changes in mitochondrial complex genes in brain samples. Red corresponds to increased gene expression, while green corresponds to decreased expression.

Table 1

Pearson correlations between tumor volume on day 26 and cytokine mRNA expression in tumor-bearing mice (n=14). An adjusted p-value of 0.006 was set to adjust for multiple comparisons.

		Tumor Volume	
		Correlation coefficient	**p-value**
Tumor	IL-6	0.577	0.031
	IL-1β	0.548	0.042
	TNF-α	0.037	0.901
Liver	IL-6	0.911	**<0.001**
	IL-1β	0.842	**<0.001**
	TNF-α	0.951	**<0.001**
Brain	IL-6	0.594	0.025
	IL-1β	0.711	0.004
	TNF-α	0.217	0.456

Table 2

Summary of altered mitochondrial gene expression in the liver and brain by complex. The percent of significantly altered genes ($p<0.05$) in each complex is represented by arrows. ↑ indicate an upregulation, while ↓ indicates a downregulation. Increasing number of arrows indicate increased number of genes significantly dysregulated: 3 arrows indicate 50-100%, 2 arrows indicate 25-49.9%, 1 arrow indicate 12.5-24.9%, and no arrows indicate <12.5% of genes significantly altered.

		Complex				
		I	II	III	IV	V
Liver	No Tumor -CRT				↓	
	Tumor-No CRT	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓
	Tumor -CRT					
Brain	No Tumor -CRT	↑		↑		↑
	Tumor-No CRT	↑↑		↑↑↓	↑	↑
	Tumor-CRT	↑↑	↑↑	↑↑↑↓↓	↑↑	