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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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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.

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

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

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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 of chemotherapy-induced symptoms, including mitochondrial dysfunction, have been proposed as potential [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 h 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 g 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.