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Tumor growth increases neuroinflammation, fatigue and depressive-like behavior prior to alterations in muscle function

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ABSTRACT

Cancer patients frequently suffer from fatigue, a complex syndrome associated with loss of muscle mass, weakness, and depressed mood. Cancer-related fatigue (CRF) can be present at the time of diagnosis, during treatment, and persists for years after treatment. CRF negatively influences quality of life, limits functional independence, and is associated with decreased survival in patients with incurable disease. Currently there are no effective treatments to reduce CRF. The aim of this study was to use a mouse model of tumor growth and discriminate between two main components of fatigue: loss of muscle mass/function and altered mood/motivation. Here we show that tumor growth increased fatigue- and depressive-like behaviors, and reduced body and muscle mass. Decreased voluntary wheel running activity (VWRA) and increased depressive-like behavior in the forced swim and sucrose preference tests were evident in tumor-bearing mice within the first two weeks of tumor growth and preceded the loss of body and muscle mass. At three weeks, tumor-bearing mice had reduced grip strength but this was not associated with altered expression of myosin isoforms or impaired contractile properties of muscles. These increases in fatigue and depressive-like behaviors were paralleled by increased expression of IL-1β mRNA in the cortex and hippocampus. Minocycline administration reduced tumor-induced expression of IL-1β in the brain, reduced depressive-like behavior, and improved grip strength without altering muscle mass. Taken together, these results indicate that neuroinflammation and depressed mood, rather than muscle wasting, contribute to decreased voluntary activity and precede major changes in muscle contractile properties with tumor growth.

1. Introduction

Fatigue is the most common symptom reported by cancer patients before and during treatment, and can continue for years after completion of treatment (Bower and Lamkin, 2013; Husson et al., 2013; Minton et al., 2012). It often co-occurs with depression, (Bower et al., 2011; Kim et al., 2012; Pertl et al., 2013) and reduces quality of life (Vissers et al., 2013). However, the cause of cancer-related fatigue (CRF) is unknown (Berger et al., 2012) and there are no effective treatments (Bower and Lamkin, 2013).

Mounting evidence indicates that CRF and depressed mood are associated with elevated serum levels of pro-inflammatory mediators, including C-reactive protein (Pertl et al., 2013) and cytokines such as tumor necrosis factor-alpha (TNFα), interleukin (IL)-1β and IL-6 (Saligan and Kim, 2012; Wood and Weymann, 2013). These cytokines are likely produced by the tumor and host tissues in response to tumor growth or anti-tumor treatments (Wang et al., 2012). Pro-inflammatory cytokines increase expression of biomarkers of autophagy and the ubiquitin–proteasome pathway in skeletal muscle which reduce muscle mass (Fearon et al., 2012; Sandri, 2013; Toledo et al., 2011). The loss of muscle mass, or sarcopenia, can be seen even before cancer treatment (Baracos et al., 2010; Cao et al., 2010) and likely explains patient complaints of exhaustion associated with physical activity and muscle weakness (Hofman et al., 2007).

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Systemic increases in pro-inflammatory mediators mount a complex response that is not limited to the periphery. The central nervous system (CNS) interprets inflammatory responses that originate in the periphery. Microglia, innate immune cells of the CNS, contribute to the propagation of inflammatory cytokines and secondary messengers throughout the CNS (Wood and Weymann, 2013). Increases in brain IL-1β are linked to both muscle atrophy (Braun et al., 2011) and depressed mood (Haroon et al., 2012). Recent evidence from rodent models indicates that inflammatory cytokines within the CNS are associated with symptoms of fatigue, such as decreased voluntary wheel running activity (Carmichael et al., 2006). Although a link between inflammation and fatigue in cancer patients has been suggested (Bower, 2007), no clear connection between CNS inflammation and CRF has been reported.

The aim of this study was to discriminate between loss of muscle mass and depressed mood in a mouse model of CRF. Fatigue was modeled as reduced voluntary wheel running activity (VWRA) (Novak et al., 2012; Wood et al., 2006; Zombeck et al., 2013) and weakness was modeled as reduced forelimb grip strength (Murphy et al., 2012). Depressed mood was modeled using the sucrose preference (Lamkin et al., 2011) and forced swim tests (Pyter et al., 2009). We show that depressive-like behavior and brain cytokine expression were increased and VWRA was decreased in tumor-bearing mice prior to the loss of muscle mass and decrease in grip strength. Decreased grip strength, however, was not associated with reduced contractile properties of skeletal muscle. Administration of minocycline to tumor-bearing mice reduced inflammatory cytokine expression in the brain, reduced depressive-like behavior, and increased grip strength with no effects on muscle mass. These data indicate that grip strength may reflect motivation or mood as much as muscle strength. Overall, decreased physical activity and depressive-like behaviors are mediated by pro-inflammatory cytokine expression in the brain of tumor-bearing mice.

2. Materials and methods

2.1. Mice

Adult female BALB/c × DBA/2 F1 (CD2F1) adult (10 weeks) mice weighing 20–22 g were obtained from Charles River Laboratories. Female mice were used because we and others have shown that tumor-bearing females maintain their food intake and lose a smaller percent of body mass than male mice (Cosper and Leinwand, 2011) and male mice often gnaw and bite at the tumor site (Yang et al., 2014). Mice were housed 1–3 per cage and maintained at 25 °C under a 12 h light cycle with *ad libitum* access to water and rodent chow. All procedures were performed in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and were approved by The Ohio State University Institutional Animal Care and Use Committee.

2.2. Mouse model of tumor-growth

The colon26 adenocarcinoma (colon26) cell line was maintained in culture and prepared for injection as previously described (Xu et al., 2011). Mice were injected subcutaneously between the scapulae with 5×10^5 cells in 0.2 ml of PBS or PBS alone. This tumor cell line secretes IL-6 and TNF-α (Graves et al., 2006) and does not metastasize when injected subcutaneously (Okayama et al., 2009). Tumor growth is usually palpable by day 7 and mice become moribund by day 24 of tumor-growth. In the present study, all data collection was completed by day 21 of tumor growth. Body mass and food and water intake were monitored three times a week for the first 2 weeks, and daily during the 3rd week. Behavioral data were collected in the range of 1 week (7 day), 2 weeks (12–14 day) and 3 weeks (19–21 day) following tumor cell inoculation. Except as noted below, mice were euthanized by inhalation of CO_2 gas and blood was withdrawn by cardiac puncture. Hindlimb muscles were dissected, weighed, and snap frozen in liquid nitrogen until biochemical analyses; tumor mass was removed and weighed; the brain was quickly dissected and hippocampus and cortex brain tissue were snap frozen in liquid nitrogen.

2.3. Oral minocycline administration

Mice were housed 3 per cage for the minocycline study. Mice were provided bottles of water or water supplemented with 1 mg/ml minocycline for a dose of 100 mg/kg/day (Sigma, St. Louis) starting one day after PBS or tumor cell injection. Water bottles were changed every other day throughout the study. There were no differences in total fluid intake between any of the experimental groups (Control-minocycline, Control-vehicle, Tumor-minocycline, and Tumor-Vehicle) (data not shown).

2.4. Grip strength measurements

Forelimb grip strength was determined as previously described (Murphy et al., 2012). In brief, each mouse was allowed to grasp a platform with both forelimbs and was pulled by the tail until it released itself from the platform (Columbus Instruments, model 1027DSM). Peak force measurements (*N*) were recorded in five trials and the average was calculated. Because smaller mice have smaller grip strength, peak force was also normalized to body mass of the animal.

2.5. Voluntary wheel running activity

Fatigue-like behavior was determined using voluntary wheel running activity as previously described (Zombeck et al., 2013). Mice were singly housed for studies in which voluntary wheel running activity (VWRA) was determined. Mice were acclimated to a four inch running wheel in the cage for one week, and baseline measures (week 0) of VWRA were recorded overnight prior to injection with tumor cells or PBS. Wheels were again placed in the home cages of all mice overnight on days 7 (week 1), 14 (week 2) and 19 (week 3) of tumor growth and the total number of turns was digitally recorded (Columbus Instruments, model 0297-004M).

2.6. Home cage locomotor activity

Mice were maintained in their home cage with a floor area of 26 × 20 cm, and activity was video recorded for 3 min. On the video records, cages were divided into 6 identical virtual rectangles and the number of line crossings was determined.

2.7. Depressive-like behavior

Depressive-like behavior was determined using resignation in the forced swim test (FST) and anhedonia in the sucrose preference test, as described previously (Godbout et al., 2008; Henry et al., 2008). In the FST, mice were placed in an inescapable cylinder (diameter 16 cm, height 30 cm) containing 15 cm of water and behavior was recorded for 5 min. The latency to become immobile and the duration of immobility were determined. For the sucrose preference test, mice were provided two solutions: water or water supplemented with 2% sucrose. Mice were fluid- and food-deprived for 2 h prior to testing. At the start of the dark phase of the photoperiod, plain water and the sucrose water were both