Project title

To determine the differential effects of tumour-derived factors (tumourkines) in the pathogenesis of cancer cachexia.

Project summary

Cachexia is a devastating condition characterised by a progressive loss of skeletal muscle and fat, leading to significant reductions in mobility and functional independence [1]. In advanced cancers, up to 80% of patients suffer from cachexia and, remarkably, 25% of cancer-related mortalities (two million people globally in 2012) are due to cachexia rather than direct tumour burden [2]. Despite this, treatment options for cachexia are lacking and patients generally receive little more than palliative care. Thus, there is a pressing need to identify the mediators of cancer cachexia and develop therapies to treat this debilitating condition.

Over the past 30 years, two groups of proteins, proinflammatory cytokines and TGF-β family members, have increasingly associated with the loss of muscle and adipose tissue in cachexia. Amongst proinflammatory cytokines, interleukin (IL)-6 is, perhaps, the best-characterised mediator of cachexia [3, 4]. Many cancer types secrete IL-6 and increased circulating levels of this cytokine in patients correlates with weight loss and reduced survival [5]. Serum IL-6 levels are also elevated in most experimental models of cachexia, and blocking IL-6 signalling can reduce the rate of cachexia in murine models [6, 7].

TGF-β proteins, particularly myostatin and activins, have been strongly linked to muscle wasting in cachexia [8, 9]. Muscle-derived myostatin and circulating activins normally act in concert to negatively regulate muscle mass[10], as such systemic elevation of these growth factors, either in the presence or absence of tumours, results in marked muscle atrophy and cachexia [8, 9, 11]. Strikingly, in cancer cachexia models, blockade of the myostatin/activin pathway (via delivery of soluble activin type II receptor (ActRIIB)) reverse muscle wasting and prolongs survival [12].

Increasing evidence signifies IL-6 and activin A as the key mediators of cachexia in murine models. In combination, studies suggest that tumours secrete multiple cachectic factors, each of which contribute to systemic wasting [4, 13]. However, because each cancer type will produce a defined set of 'tumourkines' [4], it has proven difficult to determine the relative contribution of these factors to cachexia progression. To address this, here we used an adeno-associated viral gene delivery model to look at the contribution of various tumourkines in the pathogenesis of cachexia. Simultaneously, we sought to generate and validate specific antagonists for lead candidate tumourkines, including activin A and activin B, by modification of their naturally affiliated prodomains.

Study 1: Establishing a new approach to identify the mediators of cancer cachexia.

In this study, we utilised recombinant adeno-associated viral vectors (AAV) to systematically elevate recognised tumourkines in wild type mice (i.e. in the absence of tumour burden). Though our study focussed on increasing the serum levels of IL-6 and/or activin A, this system is readily adaptable to any combination of tumourkines and enables us to determine the contribution of each factor to the pathogenesis of cachexia.

In this study, mice with elevated levels of IL-6 exhibited 8.1% weight loss after nine weeks, whereas mice with elevated levels of activin A lost 11% of their body weight. Co-elevation of both tumorkines to levels approximating those observed in cancer cachexia models induced a more rapid and profound body weight loss of 15.4%, Analysis of body composition revealed that activin A primarily triggered loss of lean mass, while IL-6 was a major mediator of fat loss [14].

The wasting derived from both co-elevation of IL-6 and activin A suggests an additive rather than synergistic effect, highlighting other tumourkines are necessary to induce severe cachexia. This study presents a useful model to deconstruct cachexia, opening a pathway to determining which tumorkines are best targeted to slow/reverse this devastating condition in cancer patients.

The findings from this study led to the following publication:

JL Chen, KL Walton, H Qian, TD Colgan, A Hagg, MJ Wwatt, CA Harrison and P Gregorevic **2016**. *Differential effects of interleukin-6 and activin A in the development of cancer-associated cachexia*, Cancer Research, 76(18): 5372-5382.

Study 2: Using specific TGF-β antagonists to prevent/reverse wasting in a cachexia model

As myostatin and activin A are the major TGF-β proteins that negatively regulate muscle mass, we propose that specifically inhibiting their actions (via modified prodomains) will limit cachexia in tumour-bearing mice. This approach should suffer from fewer side effects than were observed in clinical trials with the broad-spectrum TGF-β antagonist ActRIIB. When individually delivered into the tibialis anterior (TA) muscles of wild type mice via AAVs, the modified prodomains increased muscle mass by up to 45%. Interestingly, inhibiting both activins and myostatin/GDF11 signalling resulted in a synergistic effect, with muscle mass increasing by 156%.

In this study, we tested these reagents in the colon-26 (C26) murine cancer cachexia model, the typical model to study this disease. At the experimental endpoint (18 days), the TA muscles of C26 tumour-bearing mice exhibited a 28% reduction in mass compared with muscles of sham mice. Although inhibiting activin A and B specifically within the TA muscle had no effect, blocking myostatin/GDF11 partially reversed C26 tumor-induced wasting. Importantly, inhibiting all four ligands not only prevented muscle wasting in the C26 model, but also led to a small increase in muscle mass compared to tumour-free controls.

This study highlights the potential therapeutic advantages of targeting multiple TGF- β ligands in disorders associated with skeletal muscle wasting.

The findings from this study led to a manuscript that is currently in the rebuttal process with PNAS journal.

Manuscripts under review:

JL Chen, KL Walton, A Hagg, T Colgan, K Johnson, H Qian, P Gregorevic and CA Harrison. *Specific targeting of TGF-β family ligands demonstrates distinct roles in the regulation of muscle mass in health and disease,* PNAS.

Publications relating to this award:

JL Chen, KL Walton, H Qian, TD Colgan, A Hagg, MJ Wwatt, CA Harrison and P Gregorevic **2016**. *Differential effects of interleukin-6 and activin A in the development of cancer-associated cachexia,* Cancer Research, 76(18): 5372-5382.

Presentations relating to this award:

1) Servier Award Lecture: ESA-SRB-ANZBMS Annual Meeting, Gold Coast, QLD, Australia

> **JL Chen**, KL Walton, H Qian, TD Colgan, A Hagg, MJ Watt, CA Harrison and P Gregorevic

2) Symposium session: US Endocrine Society Annual Meeting, Boston, MA, USA.

> **JL Chen**, KL Walton, H Qian, TD Colgan, A Hagg, MJ Watt, P Gregorevic and CA Harrison

3) Poster session: Metabolic Diseases Conference, Melbourne, VIC, Australia.

> **JL Chen**, KL Walton**,** H Qian, TD Colgan, A Hagg, MJ Watt, CA Harrison and P Gregorevic.
>
> *Awarded FEBs Journal prize for best poster.

4) Poster session: International BMP Conference, Boston, MA, USA.

> **JL Chen**, KL Walton, A Hagg, TD Colgan, KE Johnson, H Qian, P Gregorevic and CA Harrison.

Planned presentations relating to this award in 2017:

- ESA-SRB ASM, Perth, WA, Australia

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