2-01

Autocrine activin A signalling regulates secretion of interleukin 6, autophagy, and cachexia

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Our understanding of the underlying mechanisms that cause cancer cachexia is limited. Several factors and inflammatory mediators released from the tumour have been suggested to contribute to weight loss in cachectic patients. However, inconsistencies between studies are recurrent. Activin A and interleukin 6 (IL-6) are among the best studied factors that seem to be important. Several studies support their individual role in cachexia development. We show that activin A acts in an autocrine manner to promote the synthesis and secretion of IL-6 from cancer cells. By inhibiting activin A signalling, using biological, chemical, or genetic approaches, the production of IL-6 from the cancer cells is reduced by 40–50%. Inhibiting activin signalling also reduces the ability of the cancer cells to accelerate autophagy in non-cancerous cells (up to 43% reduced autophagy flux). In line with the *in vitro* data, the use of an anti-activin receptor 2 antibody in cachectic tumour-bearing mice reduces serum levels of cancer cell-derived IL-6 by 62%, and importantly, it reverses cachexia and counteracts loss of all measured muscle groups. Our data support a functional link between activin A and IL-6 and indicate that interference with activin A-induced IL-6 secretion from the tumour has therapeutic potential for cancer-induced cachexia.

2-02

The mechanical stimulation of myotubes counteracts the effects of tumour-derived factors through IL-4 secretion and the modulation of the activin/follistatin ratio

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Exercise counteracts cachexia, but it is unclear to which extent the exercise-dependent mechanical stimulation of muscle *per se* plays a role in exercise beneficial effects. To study the mechanisms underlying mechanical stimulation, we cultured C2C12 myotubes in the absence or in the presence of a cyclic mechanical stretching stimulus (MS) and in the absence or presence of C26 tumour-derived factors (C26-CM), so as to mimic the mechanical stimulation of exercise and cancer cachexia, respectively. We found that C26-CM contains activin and induces activin release by myotubes, further exacerbating its negative effects, consisting in myotube atrophy and in hampering myoblast recruitment and fusion into myotubes. A high level of circulating activin is an adverse prognostic factor in cancer patients, and our *in vitro* results demonstrate that activin may be a direct player and not just a marker of cachexia. We also found that MS is sufficient to counteract the adverse tumour-mediated effects on muscle cells, in association with an increased follistatin/activin ratio in the cell culture medium, indicating that myotubes actively release follistatin upon stretching. In addition, MS induces IL-4 secretion by muscle cells. Recombinant follistatin counteracts C26 tumour effects on myotubes exclusively by rescuing fusion index, while recombinant IL-4 ameliorates fusion index, as well as the myotube size, both in terms of myotube diameter and number of nuclei per myotube. Our results indicate that tumour cells negatively affect muscle cells by releasing soluble factors and that MS is sufficient to counteract these effects, by affecting the muscle secretome

with autocrine/paracrine pathways. Activin and Act-R ligands are becoming increasingly important as triggers of muscle wasting and as pharmacological targets to treat cachexia; however, since follistatin alone is incapable to entirely block the C26-CM effects, the development of novel activin-targeted approaches should consider the existence of further significant tumour-secreted factors mediating cachexia.

2-03

Generation of reporter cell lines to identify and characterize cachexia-inducing factors

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Introduction: Cancer cachexia is mainly characterized by weight loss attributed to loss of muscle and adipose tissue, which can be induced by multiple factors, either derived from the tumour or the host. Many of these factors have been identified; however, more still remain to be discovered. In cancer cachexia, some genes are constitutively activated in target tissues, such as MuRF1 (muscle RING finger-containing protein 1) and atrogin-1 in muscle, CIDEA (cell death-inducing DFFA-like effector A) in adipose tissue. Utilizing promoters from MuRF1, Atrogin-1, and CIDEA, we have generated reporter cell lines that are capable of detecting cachexia-inducing factors released by tumour.

Methods: The promoters of genes encoding MuRF1, Atrogin-1, and CIDEA were cloned into a vector which drives the reporter genes, luciferase and GFP. Constructs were stably integrated into C2C12 myoblasts and 3T3-L1 pre-adipocytes, which when differentiated into myotubes and white adipose adipocytes, express receptors for soluble cachexia-inducing factors. Cells were differentiated and tested using various cachexia-inducing factors.

Results: Dexamethasone, activin A, and myostatin can activate C2C12 myotubes reporter cells. Thiazolidinediones can activated 3T3-L1 adipocytes. Similarly, serum from cachectic mice was also able to activate these reporter cells, compared to serum from non-cachectic mice which could not.

Conclusions: These reporter cell lines can react to the stimulation of cachexia-inducing factors. Additionally, they can also be used as a potential diagnostic tool by detecting cachectic factors in the serum or plasma of animals and patients.

2-04

Cachexia induced by non-bone metastatic cancers is accompanied by bone, cartilage, and bone marrow destruction

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Background: Cancer-induced cachexia resulting in defects in skeletal muscle mass and function is well described, although little is known about the effects of non-bone metastatic cancer on bone, particularly in the absence of metastases. In this study, we investigated the effects of two well-characterized models of cancer cachexia, namely, the mice bearing C26 adenocarcinoma and ES-2 ovarian cancer.

Methods and Results: Even though both C26 and ES-2 tumours resulted in comparable body and muscle wasting, the ES-2 hosts showed severe bone loss by microCT analysis, whereas only a modest bone loss was observed in the C26-bearing mice. Histomorphometry analysis showed increased osteoclast numbers in femoral trabecular bone from ES-2 hosts, while no significant effects were observed in the C26-bearing mice at time of sacrifice. Von Kossa staining of femur sections showed severe reduction of organized osteoblasts as well as osteoid of trabecular bone. Interestingly, both models showed dramatic increase in osteocyte death and empty lacunae, likely due to secreted tumour factors. This was also validated by *in vitro* experiments showing increased death of MLO-Y4 osteocyte-like cells when exposed to ES-2 or C26 conditioned media. Notably, dramatic depletion of fat vacuoles within the bone marrow, as well as dystrophic mineralization, likely consequence of massive bone marrow cell death, were observed in both models. In addition, the growth plate was also severely affected showing absence of hypertrophic chondrocytes and cartilage degeneration zone in both tumour models.

Conclusions: Here, we showed for the first time that bone destruction accompany muscle depletion in two models of non-metastatic cancer cachexia. Overall, this study suggests the importance of monitoring bone quality in cancer patients, even in the absence of bone metastases. Moreover, our ongoing studies will define whether anti-resorptive treatments will preserve bone mass while also improving muscle size and function in cachexia.