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METABOLISM

Ataxin-10 is part of a cachexokine cocktail triggering cardiac metabolic dysfunction in cancer cachexia

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ABSTRACT

Objectives: Cancer cachexia affects the majority of tumor patients and significantly contributes to high mortality rates in these subjects. Despite its clinical importance, the identity of tumor-borne signals and their impact on specific peripheral organ systems, particularly the heart, remain mostly unknown.
Methods and results: By combining differential colon cancer cell secretome profiling with large-scale cardiomyocyte phenotyping, we identified a signature panel of seven "cachexokines", including Bridging integrator 1, Syntaxin 7, Multiple inositol-polyphosphate phosphatase 1, Glucosidase alpha acid, Chemokine ligand 2, Adamts like 4, and Ataxin-10, which were both sufficient and necessary to trigger cardiac atrophy and aberrant fatty acid metabolism in cardiomyocytes. As a prototypical example, engineered secretion of Ataxin-10 from non-cachexia-inducing cells was sufficient to induce cachexia phenotypes in cardiomyocytes, correlating with elevated Ataxin-10 serum levels in murine and human cancer cachexia models.
Conclusions: As Ataxin-10 serum levels were also found to be elevated in human cachectic cancer patients, the identification of Ataxin-10 as part of a cachexokine cocktail now provides a rational approach towards personalized predictive, diagnostic and therapeutic measures in cancer cachexia.

Keywords Cancer cachexia; Ataxin-10; Cardiac dysfunction; Fatty acid metabolism

1. INTRODUCTION

Cancer-induced cachexia describes a multi-factorial disease condition characterized by massive loss of adipose tissue and skeletal muscle mass and is believed to be responsible for up to 30% of cancer-related deaths in humans [1]. However, the exact causes of death and in particular the contribution of cardiac dysfunction to deaths of these patients is unclear [2]. Due to the phenotypic heterogeneity of cancer cachexia, which depends on tumor type, size and mass [3], and the mostly unknown etiology at the molecular level, cachexia still represents an immediate unmet medical need as effective and routine therapeutic measures are still lacking to date [4].
Interestingly, the clinical severity of cancer cachexia hardly correlates with tumor mass [5], indicating that the tumor controls peripheral energy balance in critical host tissues via distinct signaling mediators rather than acting as a direct "energy sink" [6]. Indeed, classical experiments demonstrated the para-biotic transfer of cachexia in rats,

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indicating that circulating factors, rather than local tumor effects, act as causative factors in energy wasting [7].

Since the discovery of tumor necrosis factor (TNF) alpha as the wasting-associated “cachectin” [8], numerous cytokines have been discussed as pro-cachectic mediators, most notably Interleukin (IL) 6 [9]. In addition, zinc-alpha-2-glycoprotein (ZAG), also known as LMF (lipid mobilizing factor), has been identified as an adipokine with direct lipolytic action in white adipose tissue during murine and human cancer cachexia [10]. However, attempts to overcome cachectic energy wasting by therapeutically targeting these pathways remained rather disappointing and/or not feasible [11]. For example, administration of anti-TNF alpha antibodies [12] did not promote weight stabilization in human clinical trials, overall suggesting that the cancer cachectic phenotype results either from the combinatorial action of various factors or from yet to be discovered, individually acting, tumor-borne mediators. Indeed, technological limitations in both appropriate read-out systems as well as specific tumor secretome detection methods have thus far prevented the rational identification of tumor-borne signals and hampered major progress in prognostic/diagnostic/therapeutic options in cancer cachexia.

Whereas the vast majority of previous studies have focused on the loss of skeletal muscle and adipose tissue as phenotypic features of cancer cachexia, recent studies suggest that other organs, i.e. the liver [13,14] and the heart [15], also play a prominent role in systemic cachexia. Indeed, cardiac failure is responsible for a substantial proportion of cancer cachexia-induced deaths [16], and classical clinical studies by Burch and colleagues already documented the presence of atrophic hearts and impairment in cardiac function in cachectic tumor patients [17]. In this respect, cancer-induced cachexia in mice was found to disrupt myocardial structure and re-activate the fetal gene program [18,19], correlating with hypo-innervation in the left ventricle [20], overall suggesting that cardiac dysfunction may represent an understudied component of systemic cancer cachexia.

Here we develop and apply cellular high throughput cardiomyocyte phenotyping to test for the cachexia-inducing capacities of a newly defined set of tumor-borne signaling mediators. These cachexokines were found to be both necessary and sufficient to trigger cardiac atrophy and metabolic dysfunction in cardiomyocytes. As a prototype, Ataxin-10 was found to be sufficient to cause cachectic cardiac phenotypes and to signal the existence of cachexia by its serum levels in murine and human model systems as well as in pancreatic cancer patients.

2. METHODS

2.1. Chemicals

All chemicals were purchased from Sigma Aldrich (Munich, Germany) unless stated otherwise. Cell culture media and supplements were from Life Technologies (Darmstadt, Germany) unless stated otherwise. Cell culture plastic ware was obtained from BD (Heidelberg, Germany) unless stated otherwise.

2.2. Animal experiments and treatments

Ectopic mouse model: 9—10 week old male Balb/c, C57BL6/N and Fox Chase SCID mice were obtained from Charles River Laboratories (CRL, Brussels, BE). Mice were injected subcutaneously into the right flank with 1.5×10^6 C26 colon carcinoma cells, 5×10^5 MC38 colon carcinoma cells and 5×10^6 SW480 cells, respectively. C26 mouse experiment: Mice were monitored for 21 days after tumor cell implantation and a cohort of mice (6 control and 10 tumor-bearing mice) was sacrificed every week after Pressure-Volume loop measurement. Echocardiography and Pressure-Volume loop (PV loop) measurement were performed weekly to assess the cardiac performance. Body composition was determined by Echo Magnetic Resonance Imaging (EchoMRI; Echo Medical Systems, Houston, TX, USA) analysis once a week. Body weight and food intake were determined in regular intervals. MC38 mouse experiment: Mice were monitored for 30 days after tumor cell inoculation. Cardiac function was assessed by weekly echocardiography. Body composition was also measured once a week. Body weight and food intake were determined in regular intervals. SW480 mouse experiment: Mice were monitored until the onset of cachexia (15—21 days after tumor cell inoculation). Body weight was measured at regular intervals.

Orthotopic mouse model (PDAC): 10 week old male C57BL6/J mice were obtained from Charles River Laboratories (CRL, Brussels, BE). Anesthetized mice treated with 5 mg/kg Carprofen as analgesia were injected with 3000 cells (PDAC cell line #8024 or #8025; kind gift from Dieter Saur, TU Munich, Germany) into the pancreas. Mice were monitored until the onset of cachexia (24—26 days after tumor cell inoculation). Body composition was determined once a week. Body weight was measured in regular intervals.

Genetic mouse model (APC delta 580 mice): APC delta 580 mice on a C57BL6/N background were originally purchased from the National Cancer Institute (NCI) at Frederick (Frederick, MD, USA) and bred at the Animal Facility of the German Cancer Research Center (DKFZ; Heidelberg, Germany). Heterozygous APC delta 580 male mice and age-matched wild-type C57BL/6N mice with an age of 14 weeks were monitored until the onset of cachexia (at 4—6 months of age). Cardiac performance which was measured by echocardiography and body weight were determined in regular intervals.

Diabetic mice: 9—11 week old female ob/ob and db/db mice were obtained from Charles River Laboratories (CRL, Brussels, BE). After 1 week, mice were sacrificed in random fed state.

A detailed description of general animal handling, hemodynamic measurement techniques and histology can be found in the supplemental material. The animal care and all experimental protocols were reviewed and approved by local authorities (Regierungspräsidium Karlsruhe, Germany; G23/08; G12/06; G73/11; G178/13).

2.3. Cell culture

Cell lines were regularly tested for mycoplasma contamination by the company Multiplexion using Multiplex Cell Contamination Test (McCT) [21].

2.4. Preparation of conditioned supernatants (SN)

C26, MC38 (kind gift from C. Bourquin, Clinical Pharmacology, Munich, Germany) and HEK293A cells (ATCC, Manassas, VA, USA) were plated onto 15 cm plates and after reaching a confluency of ∼50% culture medium (DMEM high glucose, 10% FBS, 1% Pen/Strep) was changed. After 48 h, media were collected and the remaining cells were pelleted by centrifugation. For the treatment of cardiomyocytes, conditioned SN was 3:1 diluted in fresh culture medium. A detailed description of used transfection procedures can be found in the supplemental material.

2.5. Primary neonatal rat cardiomyocytes

Hearts were isolated from male and female Wistar rats at the age of 1—2 days. Heart slices were digested in 0.1% pancreatin. To reduce the amount of non-cardiomyocyte like epithelial cells and fibroblasts, cells were pre-plated on 15 cm plates. Afterward, cells were harvested, counted and finally plated on cell culture plates that were coated with 0.1% gelatin (BD, Heidelberg, Germany). Cells were cultured in DMEM high glucose supplemented with 10% FBS and 1% Pen/Strep. After 3 days of plating cells were treated with conditioned SN for 24 h. A detailed description of the high throughput analysis of cardiomyocyte size and immunofluorescence staining can be found in the supplemental material.