Cytokines as molecular biomarkers for cancer cachexia

Claudia A. Penafuerte[1], Michel L Tremblay[1], Jacinthe Sirois[1], Lin Yang[2], Jessica Murphy[2], Giovanni Arcuri[2] and Bruno Gagnon[2].
Affiliations: 1 Rosalind & Morris Goodman Cancer Research Centre, McGill University and 2 Health Centre – Division of Clinical Epidemiology, McGill University Montreal Canada

Abstract

Cachexia is a multifactorial syndrome defined by irreversible loss of skeletal mass (with or without loss of fat mass) that cannot be reversed by conventional nutritional support. Cachexia is characterized by a negative protein and energy balance that causes disorders in homeostasis such as progressive wasting, weakness, anorexia and anemia. This life-threatening syndrome is present in ~30-50% of cancer patients and is markedly associated with lung and upper gastrointestinal cancers such as pancreatic cancer. In fact over 80% of pancreatic cancer patients develop cachexia, and they are more likely to have an increased progression of their tumors and their metastasis. In the present clinical study, we have analyzed and correlated the cytokine profile and muscle function data of 71 patients with advanced cancer including head and neck cancer (13 patients), Non-small cell lung cancer (NSCLC, 10 patients), pancreatic cancer (32 patients), hepatobilliary carcinoma (6 patients), colorectal cancer (8 patients) and upper gastro-intestinal cancer (GI, 2 patients). Human blood samples were drawn into anticoagulant EDTA-coated collection tubes and 16 cytokines and chemokines including IL-1β, IL-4, IL-6, IL-10, TNFα, TRAIL, IFNγ, TGFβ(1,2,3), MCP1, IL-1α, IL-15, IL-8, IL-18 and IL-12 in plasma were measured using Human Cytokine bio-plex technology. Patients with impaired muscle function are characterized by elevated levels of IL-1β, IL-6, IL-4, IFNγ, CRP (C-reactive protein) and LDH (lactate dehydrogenase). In particular, IL-1β, IL-6 and IFNγ may trigger the NF-kB mediated protein degradation pathway in the muscle and impair myoblast survival, proliferation and differentiation by acting as inhibitors of IGF induced PI3K/AKT pathway. Understanding the molecular mechanism of muscle wasting not only will identify potential targets to treat this syndrome, but also will increase the quality of life for both patients and family, which will allow them to pursue existing and upcoming treatments for cancer.

Introduction

Cancer cachexia is defined as a complex metabolic disorder associated with underlying illness and characterized by loss of muscle mass with or without loss of body fat. Clinical manifestations include anemia, reduced caloric intake, ineffective host antitumor response and abnormal lipolysis. Consequently cancer patients suffer of anorexia, fatigue, decreased muscle strength, increased disability, and diminished quality of life and survival (1). This life-threatening syndrome affects more than one million people in Unite States and is more frequently associated with lung and upper gastrointestinal cancers such as pancreatic cancer (2).

Loss of muscle mass associated to cancer cachexia is a result of a combination of increased protein degradation and decreased rate of protein synthesis. There are three major proteolytic pathway involved in the degradation of protein in the muscle: 1) The lysosomal system responsible for the degradation of extracellular proteins and cell receptors, 2) The calcium-activated system (aka calpain system) involves in tissue injury, necrosis and autolysis and 3) The ubiquitin-proteasome pathway, which function in harmony with the calpain system to disassemble and degrade muscle myofilaments (3). Specifically, the induction of muscle-specific E3 ubiquitin ligases muscle atrophy F box (MAFbx/atrogin1) and muscle RING finger 1 (MuRF1) are responsible for the selective polyubiquitination of proteins target for degradation in the muscle (4,5).

Clinical and epidemiologic studies have suggested a strong association between chronic inflammation, and cancer (6). In particular, solid malignancies trigger an intrinsic undesired inflammatory response that give rise to a protumorigenic microenvironment characterized by recruitment of leukocytes and expression of tumor-promoting cytokines and chemokines (7). This chronic inflammatory state leads to an abnormal increase of inflammatory factors that induce cachexia. Proinflammatory cytokines such as IL-1β, IL-6, TNFα, and IFNγ have shown to extensively contribute to the cascade of events that trigger muscle waste and cachexia symptoms. These cytokines have been directly or indirectly implicated in the processes of protein catabolism in the muscle, inhibition of protein anabolism, increase of insulin resistance and lipolysis in cancer (8,1). Specifically IL-1β, IL-6 and TNFα induce the activation of the transcription factors NF-kB, which is one of the essential regulators of the ubiquitin-proteasome pathway. In addition, these cytokines as well as IFNγ induce insulin resistance and impair muscle function by acting as inhibitors of IGF-induced PI3K/AKT activation (4).

In the present study, we aim to correlate the cytokine profile with muscle function in cancer patients at late stage of disease progression.

Figure 1

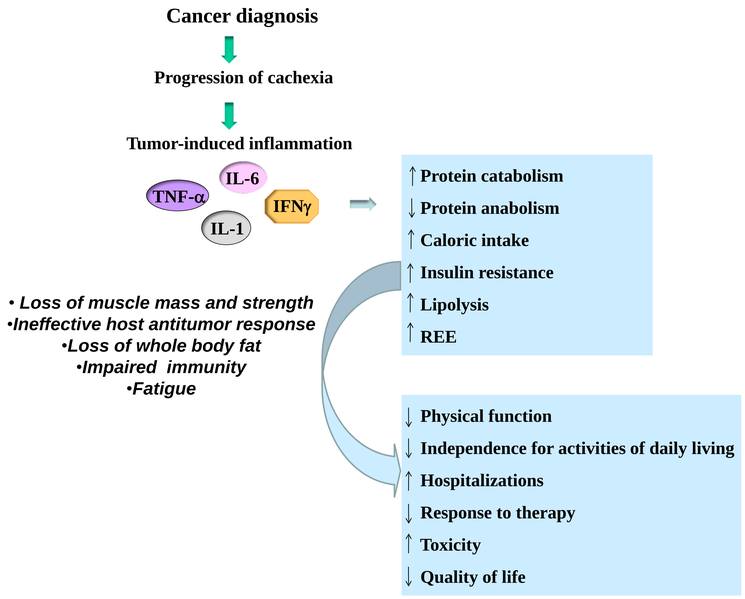
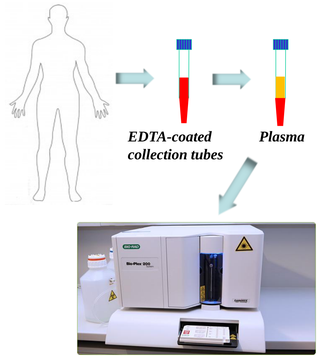


Figure 1: Cascade of events that lead to cancer cachexia and impact in the patients. Tumor-associated chronic inflammation induces an abnormal increase of proinflammatory cytokines such as IL-1β, IL-6, TNFα, and IFNγ. These cytokines have been directly or indirectly implicated in the processes of protein catabolism in the muscle, inhibition of protein anabolism, increase of insulin resistance and lipolysis in cancer. Consequently, cancer patients suffer of impaired physical functions, reduce independence for activities of daily living, reduce response to therapy and impaired quality of life.

Figure 2

Age: mean (SD)	64.8 (12.4)
Sex: n (%)	
Male	47 (66.2)
Female	24 (33.8)
Cancer Type: n (%)	
Head & Neck	13 (18.3)
Upper Gastrointestinal	2 (2.8)
Lung	10 (14.1)
Hepatobilliary	6 (8.5)
Pancreatic	32 (45.1)
Colorectal	8 (11.3)



TRAIL, IL-12, IL-18, TGFβ1, TGFβ2, TGFβ3, IL-1α , IL-5, IL-3, IL-10, TNFα, IL-8, MCP1, IFNγ, IL-1β, IL-6, IL-4.

Figure 2: Materials and Methods. Blood samples were collected from 71 patients with advanced cancer including head and neck cancer (13 patients), Non-small cell lung cancer (NSCLC, 10 patients), pancreatic cancer (32 patients), hepatobilliary carcinoma (6 patients), colorectal cancer (8 patients) and upper gastro-intestinal cancer (GI, 2 patients). Human blood samples were drawn into anticoagulant EDTA-coated collection tubes and 16 cytokines and chemokines in plasma were measured using Human Cytokine bio-plex technology.

Figure 3

Percent weight loss: n (%)*	
< 2 %	14 (25.0)
2 – 5 %	8 (14.3)
5 – 10 %	13 (23.2)
> 10 %	21 (37.5)
Skeletal muscle mass (kg): mean (SD)†	25.5 (6.7)
Two-Minute Walk Distance (m): mean (SD)‡	116 (34)
Comfortable Gait Speed (m/s): mean (SD)§	1.09 (0.32)
Maximal Gait Speed (m/s) : mean (SD)‖	1.47 (0.40)

*N = 56; †N = 55; ‡N = 57; §N =62; ‖N =61

Figure 3: Statistical analysis of muscle mass and function: Number and percentage of patients based on the percentage of weight loss. Mean values and standard desviation (SD) were obtained by measuring three muscle functions: two minutes walking distance, comfortable gait speed and maximal gait speed. The N values indicate the number of patients tested per muscle function.

Figure 4

Figure 4: Correlation analysis between the cytokine profile and muscle function. The cytokine profile and muscle function data were correlated using the statistical method Partial Least Squares (PLS). Patients with impaired muscle function are characterized by elevated levels of IL-1β, IL-6, IL-4, IFNγ, CRP (C-reactive protein) and LDH (lactate dehydrogenase).

Figure 5

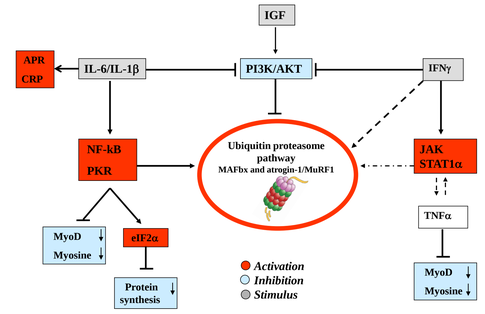


Figure 5: Underlying mechanism of muscle waste. Proinflammatory cytokines IL6 and IL-1β induce protein catabolism and inhibit protein anabolism through the activation of NF-kB and PKR signaling pathways. IL-6 also induce acute phase response (APR) characterized by elevate levels of CRP. These cytokines along with IFNγ impair myoblast survival, proliferation and differentiation and induce insulin resistance by acting as inhibitors of IGF-induced PI3K/AKT pathway and suppress the expression of MyoD and myosine heavy chain.

Figure 6

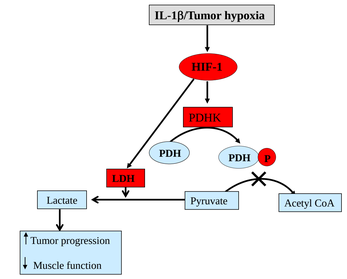


Figure 6: Mechanism of LDH induction and its effect on muscle function. IL-1β and tumor-induced hypoxia induce HIF-1 (hypoxia inducible factor 1), which increase the transcription of LDH, glycolytic enzymes and the pyruvate dehydrogenase kinase (PDHK). In turn, PDHK phosphorylate and inactivate the pyruvate dehydrogenase complex (PDH), which convert pyruvate into acethyl-CoA in mitochondria, causing the accumulation of pyruvate, which is then convert to lactate by LDH. Elevate levels of lactate increase tumor progression and impair muscle function.

References

1 Dodson,S., Baracos,V.E., Jatoi,A., Evans,W.J., Cella,D., Dalton,J.T. and Steiner,M.S. Muscle wasting in cancer cachexia: clinical implications, diagnosis, and emerging treatment strategies, Annu.Rev.Med., *62:* 265-279, 2011.
2 Bruera,E. ABC of palliative care. Anorexia, cachexia, and nutrition, BMJ, *315:* 1219-1222, 1997.
3 Hasselgren,P.O., Wray,C. and Mammen,J. Molecular regulation of muscle cachexia: it may be more than the proteasome, Biochem.Biophys.Res.Commun., *290:* 1-10, 2002.
4 Tisdale,M.J. Mechanisms of cancer cachexia, Physiol Rev., *89:* 381-410, 2009.
5 Glass,D.J. Signalling pathways that mediate skeletal muscle hypertrophy and atrophy, Nat.Cell Biol., *5:* 87-90, 2003.
6 Coussens,L.M. and Werb,Z. Inflammation and cancer, Nature, *420:* 860-867, 2002.
7 Mantovani,A., Allavena,P., Sica,A. and Balkwill,F. Cancer-related inflammation, Nature, *454:* 436-444, 2008.
8 Baracos,V.E. Cancer-associated cachexia and underlying biological mechanisms, Annu.Rev.Nutr., *26:* 435-461, 2006.

The Terry Fox Research Institute
L'Institut de recherche Terry Fox