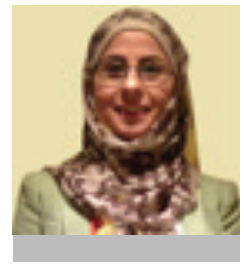

AMAL HUSSAIN IBRAHIM AL HADDAD

Department of Physiology
College of Medicine and Health Sciences

Dissertation

Title *Identifying the Molecular Mechanisms of Early Cachexia Using Whole Transcriptome Sequencing in Muscle and Fat Biopsies from Cancer Patients*

Faculty Advisor Prof. Thomas E. Adrian

Defense Date 10 June 2015

Abstract

Cachexia causes one third of cancer–related deaths and contributes to that of many others. Despite extensive research, the mechanisms of cancer cachexia are poorly understood. Identification of early changes in gene expression in the major cachexia target tissues will improve the understanding of its mechanisms. We investigated the entire transcriptome, using next generation sequencing (Illumina HiSeq 2500), to identify altered expression of genes in muscle and fat from cancer patients. Samples of rectus abdominis muscle and visceral fat were collected at surgery from patients exhibiting 5-10% weight loss prior to surgery, compared with stable-weight patients. Also, selected differentially ex-pressed genes were confirmed using real-time RT-PCR. In muscle, 30 genes showed highly significant change in expression (25 down and 5 up: P<0.0005 - P<0.00001, FDR 0.2). The 25 downregulated genes included 7 involved with metabolism (5 mitochondrial); 4 with signaling; 4 with ubiquitination; and 3 with intracellular trafficking. Multiple genes involved in glycogen metabolism were downregulat-ed, correlating with the lack of glycogen, muscle weakness, and fatigue; characteristic of cachexia. The 5 upregulated genes include 2 involved with calcium signaling and 2 with cell matrix interactions. Expres-sion of genes previously thought to be important in cachexia, including several inflammatory cyto-kines, was not significantly different. FBXO32, which encodes atrogin-1, upregulated in an in vitro cachexia model, was actually downregulated. No transcripts for the dermicidin gene, which codes for proteolysis-inducing factor, were detected. Expression of myostatin and its receptor (ACTR2B) were significantly decreased, possibly reflecting end organ adaptation to tumor produced myostatin. In visceral fat, expression of 6 genes were downregulated and 10 upregulated with high statistical signifi-cance (P<0.001-0.0002). Several of these encode metabolic enzymes. Of genes in fat previously impli-cated with cachexia, such as hormone sensitive lipase and adipose tissue triglyceride lipase, were un-changed. In contrast, leptin was significantly downregulated and the zinc-□-2-glycoprotein (lipid mobi-lizing factor) was significantly upregulated as expected. These studies explain some documented evi-dence in cachexia pathogenesis, highlight ambiguous data from animal models, and reveal unexpected changes in gene expression that underlie the pathophysiology of the cachectic state in cancer. These results bring reliable, representable, and consistent data from the clinic and back to the bench with more focused insights to be investigated and verified.

Research Relevance and Potential Impact

Most patients with cancer exhibit marked wasting of skeletal muscle and anorexia which is known as the cancer cachexia syndrome. Cachexia is a major cause of cancer death and it affects the quality of life, the response to therapy, the ability to withstand the rigors of therapy and even the psychological wellbeing of the patients and their families. The mechanisms of cancer cachexia are not well understood. We hypothesized that changes in gene expression in early cachexia would shed light on pathways involved which would then pave the way for a more targeted approach to therapeutic intervention. The entire transcriptome of skeletal muscle and visceral adipose tissue was investigated using next-generation sequencing and the major changes confirmed by fast real-time RT-PCR. These studies indicate the involvement of several metabolic pathways that explain much of the clinical observations in cancer cachexia. The data confirms some of the documented evidence in the pathogenesis of cachexia, highlights ambiguous data from animal models, and reveals unexpected changes in gene expression that underlie the pathophysiology of the cachectic state in cancer. Once confirmed in other groups of patients with cachexia these findings are likely to lead to improvements in the prevention and treatment of cachexia in patients with cancer.