The International Journal of Biochemistry & Cell Biology 50 (2014) 106–111

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Organelles in focus

Integrated transcriptome analysis across mitochondrial disease etiologies and tissues improves understanding of common cellular adaptations to respiratory chain dysfunction

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ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form 10 February 2014
Accepted 16 February 2014
Available online 22 February 2014

Keywords:
Oxidative phosphorylation (OXPHOS)
Gene expression
Microarray
RNAseq

ABSTRACT

Mitochondrial diseases are heterogeneous, multi-systemic disorders for which mechanistic understanding is limited. To investigate common downstream effects of primary respiratory chain dysfunction on global gene expression and pathway regulation, we reanalyzed transcriptome datasets from all publicly available studies of respiratory chain dysfunction resulting from genetic disorders, acute pathophysiologic processes, or environmental toxins. A general overview is provided of the bioinformatic processing of transcriptome data to uncover biological insights into *in vivo* and *in vitro* adaptations to mitochondrial dysfunction, with specific examples discussed from a variety of independent cell, animal, and human tissue studies. To facilitate future community efforts to cohesively mine these data, all reanalyzed transcriptome datasets were deposited into a publicly accessible central web archive. Our own integrated meta-analysis of these data identified several commonly dysregulated genes across diverse mitochondrial disease etiologies, models, and tissue types. Overall, transcriptome analyses provide a useful means to survey cellular adaptation to mitochondrial diseases.

1. Introduction

The mitochondrial respiratory chain (RC) functions at the core of oxidative metabolism to convert reducing equivalents generated from cellular nutrients into chemical energy in the form of adenosine triphosphate (ATP). In the process, the RC plays an essential role in establishing the reduced and oxidized (redox) balance of nicotinamide adenine dinucleotide (NAD^+), which regulates hundreds of cellular reactions and serves as an acetylation precursor. The RC also generates reactive oxygen species (ROS), whose balance can variably serve as secondary messengers to influence cell signaling or generate damage to cellular DNA, lipids, and proteins. RC function further lies at the hub of the intermediary metabolism network, cellular calcium regulation, and apoptosis. Given the wide-reaching implications of these core cellular functions, it is not unexpected that RC dysfunction will produce a host of changes across diverse aspects of cellular physiologic and metabolic processes. The challenge lies in accurately and concisely characterizing the specific nature or degree of such effects in any particular disease state, tissue, or cellular condition.

A 'transcriptome' refers to all RNA classes (mRNA, miRNA, *etc.*), forms (unspliced, degraded, *etc.*), and locations (nucleus, mitochondria, *etc.*). Transcriptome profiling has emerged as a potent means by which to investigate the downstream, or "retrograde", effects of primary RC dysfunction, as can variably result from a genetic etiology, acute pathophysiologic process, or environmental toxin. Wide-spread transcriptome analysis became possible with the advent of microarray technologies. While microarray analyses can utilize different methodologies and platforms to measure RNA abundance, they generally provide consistent results that define major differential expression (DE) between any two conditions at the levels of genes and pathways (Zhang et al., 2010). Recent progress in massively parallel sequencing technologies has now made RNAseq an even more comprehensive and sensitive tool than microarrays to simultaneously interrogate more sophisticated aspects of the transcriptome, such as alternative splice forms, allele-specific expression, and a broader dynamic range to accurately delineate low-level and high-level gene expression.

To investigate common downstream effects of primary RC dysfunction on global gene expression and pathway regulation, we reanalyzed transcriptome datasets from all publicly

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http://dx.doi.org/10.1016/j.biocel.2014.02.012
1357-2725/

available studies of RC dysfunction resulting from genetic disorders, acute pathophysiologic processes, or environmental toxins. We accessed the data from Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo/), which is the major public repository for transcriptome data. However, the reusability of GEO data has generally been impeded by inconsistent data processing and gene annotation between independent studies. To address this problem, we downloaded and subjected to a common processing and annotation protocol data from more than 30 GEO datasets that were previously generated from a wide variety of primary RC disease cell, tissue, and animal models. Each individual data set was reanalyzed to calculate gene-level DE by comparing samples that were divided into two groups, as based on their having either normal or impaired RC function. Since no results had previously been reported at the integrated metabolic pathway-level DE for many of the datasets, we also applied gene set enrichment analysis (GSEA) to investigate the overall DE of canonical metabolic pathways in each dataset (Subramanian et al., 2005). All analyzed data were deposited at a public web archive (http://goo.gl/nOGWC2), along with a complete description of the detailed data processing and analysis pipeline used. A brief overview of the major data processing and analysis steps used for transcriptome analysis is graphically depicted in Fig. 1.

2. Transcriptome profiling of cellular effects in genetic and pharmacologic-based cellular and animal models of primary mitochondrial RC function

Retrograde effects of RC dysfunction have been evaluated by transcriptome profiling in a wide range of experimental animal and cellular models that were variably generated by genetic means (*e.g.*, retroviral gene transfection or RNA interference) or chemical exposure to dysregulate the expression of individual mitochondria-localized proteins and enable identification of the downstream responses to primary RC dysfunction. However, caution must be used when interpreting transcriptome data to assess potential functional consequences of any primary metabolic deficiency. In particular, while identifying significant DE of genes or pathways is suggestive that a cellular response has been invoked, it does not clarify whether that response achieved a particular functional effect. For example, although many RC disease models produce secondary upregulation of genes directly involved in oxidative phosphorylation (OXPHOS), this transcriptional response may not necessarily be fully effective in restoring electron flux, the mitochondrial membrane potential, and ATP generation. Exploring the specific components of a differentially regulated pathway that drive a particular result is also important, as some pre-defined 'pathways' may lump together both anabolic and catabolic aspects of metabolism and potentially mask DE of that pathway toward one key direction. Thus, transcriptome profiling can implicate specific biological pathways that are dysregulated by RC disease, but alone is insufficient to determine whether a specific transcriptional response is physiologically adaptive or maladaptive.

In vivo effects of RC dysfunction have been studied by transcriptome profiling in several animal models. Models have variably used either genetic mutations in specific RC complex protein subunits or chemical compounds that directly inhibit specific RC complexes, such as rotenone (complex I (CI) inhibitor) or oligomycin (complex V (CV) inhibitor). The dosage and duration of a chemical exposure can be precisely manipulated to gain insight into RC inhibition duration, degree, and cell-type specific effects. For example, adult *C. elegans* worms exposed to rotenone for a period of 1–20 days showed a transcriptome response that was reported to be most evident after a 1 day exposure and then diminish with prolonged exposure (Schmeisser et al., 2013). Our reanalysis of that dataset confirmed that transcriptome changes following different rotenone treatment durations were poorly correlated ($r = 0.03$): the mTOR signaling pathway was significantly downregulated after 1 day of rotenone exposure but then gradually normalized with prolonged exposure; the proteasome pathway was downregulated until 10 days and then showed significant upregulation by day 20 of rotenone exposure; and ribosomal proteins stayed upregulated while WNT pathway expression stayed downregulated regardless of rotenone exposure duration. Interestingly, exposing *C. elegans* to rotenone for 20 days resulted in similar transcriptome changes as were seen in the *C. elegans gas-1*(*fc21*) strain that harbors a genetic missense mutation in a nuclear-encoded CI protein subunit (Falk et al., 2008), where both models showed significant upregulation of nutrient metabolism pathways. These findings exemplify the types of insights that may be gained into *in vivo* adaptation by studying the effects in model animals of pharmacologic RC inhibitors with varying concentration and time-course experiments.

Chemical inhibitors are also useful to characterize the *in vitro*, cell type-specific transcriptome response to primary RC dysfunction. For example, exposure of human neuroblastoma cells to two rotenone concentrations (5 nMol and 50 nMol) for 1 or 4 weeks (Cabeza-Arvelaiz and Schiestl, 2012) revealed that while the higher concentration induced a globally greater degree of DE, transcriptome responses at both concentrations were positively correlated ($r = 0.70$). Comparison of the different durations of rotenone treatment, however, revealed a globally reversed pattern of DE at 4 weeks compared to 1 week, particularly at the higher rotenone concentration ($r = -0.60$). Furthermore, unlike the attenuation in transcriptome changes that was seen in rotenone-treated adult *C. elegans* worms, cultured neuroblastoma cells responded to longer rotenone exposure with a greater degree of DE. Rotenone effects have also been studied in mouse embryonic fibroblasts (MEFs) derived from genetic models of mitochondrial disease, such as the *HtrA2* knock-out (KO) mice (Moisoi et al., 2009). Although *HtrA2* KO cells exhibited a stronger response to rotenone exposure than did normal MEFs, their global pattern of DE was positively correlated ($r = 0.60$). For example, the proteasome pathway was among the most significantly downregulated KEGG pathways by rotenone treatment in both *HtrA2* KO and control MEF cells. These results are suggestive that the specific response to rotenone-induced CI inhibition depends on cell type, underlying genetic background, duration of RC inhibition, as well as *in vivo versus in vitro* status that may reflect a variety of factors including differences in oxygen tension, nutrient availability, and tissue-specific energy demand.

3. Central signaling mediators regulate physiologic effects of mitochondrial RC dysfunction

While transcriptome response to RC dysfunction clearly is influenced by a range of variables, the identification of concordant gene changes and recurrent pathway-level DE results across independent studies and models are suggestive that a "central hub" exists that converges upstream signals from RC dysfunction to modulate downstream cellular responses. The integrated nutrient-sensing signaling network (NSSN) centered on the AKT/mTORC pathways appears to be one such central mediator of cellular response to RC dysfunction (Zhang et al., 2013). Major regulatory NSSN nodes include AMPK (low energy sensor), mTORC1 (cell growth regulator by balancing cytosolic protein synthesis and autophagy), SREBP (lipid homeostasis), *FOXO1* (glucose homeostasis), and *PPAR* family transcription factors (lipid metabolism), as well as *YY1/PGC1α* (mitochondrial ribosome biogenesis) and *HIF1α* (hypoxia response) transcription factors. The involvement of the integrated NSSN in regulating cellular response to RC dysfunction was first revealed by our recent study of skeletal muscle and fibroblasts from a