Metabolic network analysis for understanding the biology of aging

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Abstract: Aging is a complex and multifactorial process that causes progressive functional decline and decreased ability of an organism to respond to stress. Although aging is typically not considered a disease, it is a major risk factor is a plethora of human diseases with widely varying pathologies. The age-related changes on human metabolism are still incompletely understood. Nevertheless, metabolic interventions, such as dietary restriction, are known to modulate aging in several model organisms. In this study, we analyzed human post-mortem transcriptomics data from the Genotype Tissue Expression project to elucidate the specific metabolic pathways involved in the human aging process. More specifically, we used human genome-scale metabolic network model to identify metabolic subsystems that are enriched among genes whose expressions vary with age. Our analysis showed a repression of cellular metabolic processes along with the disruption of amino acid and lipid balance.

Keywords: Systems Biology, Transcriptomics, Aging, Bioinformatics

1. INTRODUCTION

Aging affects almost all living things on earth, but at varying rates. The aging process is characterized by a gradual loss of function that results in increased morbidity and ultimately culminates in death. Over the last two decades, unprecedented advances in aging research have led to the identification of hallmarks of aging, i.e. conserved biological pathways that can be modulated to affect the aging rates in model organisms (Lopez-Otin et al., 2013). While model organisms are certainly indispensable in studying the aging process, translating the discoveries to human is often challenging. The complexity of the aging process further motivates applying a systems-oriented approach through the creation and analysis of cellular networks. In this regard, recent human omics profiling efforts such as Genotype-Tissue Expression (GTEx) and Human Metabolic projects have produced a wealth of human data from different tissues and age groups, for a better understanding of human aging. The challenge now is in extracting meaningful insights from these datasets.

In this study, we focused on the connection between aging and metabolism. Dietary restriction is the most robust and successful aging intervention strategy which has been successfully confirmed in many model organisms. However, the specific metabolic pathways that are modulated during the aging process are still incompletely known. We leveraged on the human transcriptomics data from GTEx project and the curated human genome-scale metabolic network model (RECON 2) (Thiele et al., 2013), to elucidate age-related metabolic alterations in human. Briefly, we employed a linear mixed effects model to determine genes whose expression are altered with aging across different tissues and sex. Projecting these age-related gene expression changes onto the human genome-scale metabolic network, we were able to identify specific age-related perturbations. We were also able to identify aging metabolic signatures in 11 human tissues. Our findings pointed to age-related alterations in pathways pertaining to cellular energy generation, branched-chain amino acid homeostasis, fatty acid metabolism and stress response.

2. METHODS

2.1 Age-related gene expression changes

For our analysis, we obtained RNA-sequencing (RNA-seq) data from the GTEx analysis V7 (phs000424.v7.p2). The database consists of RNA-seq measurements of 56202 transcripts collected from 714 subjects whose ages range from 20 to 79 years old. Information on sample collection, quality control and normalization is available elsewhere (Mele et al., 2015). In our analysis, genes with zero expression in more than 95% of samples were excluded. We considered 49 tissues in the analysis for differential gene expression after eliminating tissues with fewer than 20 samples.

We employed a linear mixed effect model to elucidate the overall age-related gene expression changes, as follows

$$\mathrm{Y}_{ijk} = \beta_i + \alpha_{ij}\mathrm{T}_j + \gamma_{ik}\mathrm{Sex}_k + \varepsilon_i\mathrm{Age}_k + b_k + e_{ijk} \quad (1)$$

where Y_{ijk} is the expression of gene *i* in tissue *j* belonging to the subject *k*, $\alpha_{ij}T_j$ and $\gamma_{ik}Sex_k$ denote the fixed effects of tissue (T_j) and sex, respectively, and b_k is the random effect associated with each subject. To analyse tissue-specific gene expression changes, the linear mixed effect model was applied on a subset of the transcriptomic data belonging to that particular tissue, without the term $\alpha_{ij}T_j$. The number of differentially expressed genes with age (FDR<0.01), referred to below as aging genes, for 11 major tissues are tabulated in Table 1.

2.2 Metabolic alterations

RECON 2 consists of 2140 metabolic genes that control 4821 metabolic reactions. The reactions are classified into various subsystems based on their role and function. We mapped the aging genes to the human genome-scale metabolic network by identifying the metabolic reactions whose enzymes are among the aging genes (see Fig 1). Finally, we performed Fisher exact tests to determine the over-representation of different metabolic subsystems among the aging genes.

Table 1. Number of differentially expressed genes

Tissue	Number of Aging genes		
	Up	Down	Total
Overall	2968	4564	7532
Adipose	6505	816	7321
Artery	2229	2746	4975
Blood	126	3128	3254
Brain	28	7212	7240
Colon	4514	1271	5785
Esophagus	2442	1540	3982
Heart	17	435	452
Lung	1951	2446	4397
Muscle	2084	992	3076
Nerve	2996	611	3607
Skin	1342	643	1985

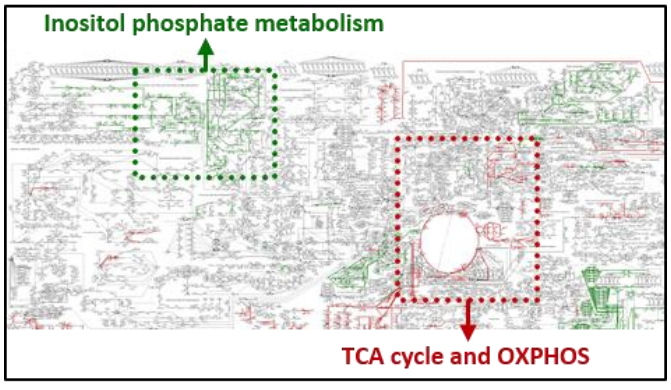

Fig. 1. Mapping the aging genes to the genome scale metabolic model allows us to look at over-represented subsystems by downregulated (highlighted in red) and upregulated (green) aging genes.

3. RESULTS

Our analysis pointed to significant age-related metabolic alterations for metabolic pathways pertaining to cellular energy generation. In particular, tricarboxylic acid (TCA) cycle and oxidative phosphorylation processes are overrepresented among the set of downregulated aging genes (see Fig 2A).

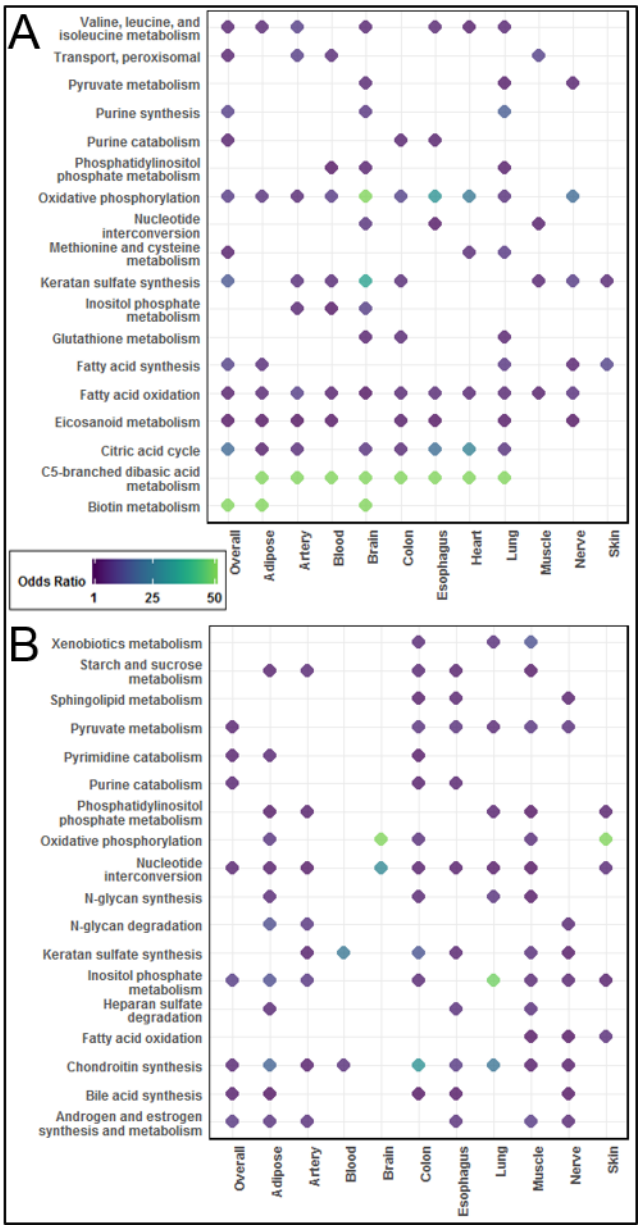

Fig. 2. Enriched subsystems across multiple tissues by (A) downregulated tissue specific aging genes and (B) upregulated tissue specific aging genes.

These pathways are also over-represented among downregulated aging genes across multiple tissues, despite the varying number of overlapping aging genes and the number of aging genes among tissues. Pathways involved in fatty acid oxidation are also downregulated across all tissues. Indeed, impaired fatty acid oxidation has previously been

shown to be associated with mouse aging (Houtkooper et al., 2011). Meanwhile, inositol phosphate metabolism pathway is enriched among upregulated aging genes across different tissues (see Fig 2B). This pathway regulates cell proliferation and is also involved in the energy sensing pathway PI3K/Akt. Our analysis also showed disruption of amino acid homeostasis (nucleotide interconversions) and branched chain amino acid metabolism.

6. SUMMARY AND OUTLOOK

Our analysis of human gene expression across age groups indicated cellular energetic crisis accompanying the aging process in human. Besides energy production, our analysis further showed disruptions in protein homeostasis during human aging. The analysis and understanding of metabolic connections with aging is an important step toward formulating a strategy for mitigating aging.

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