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RESEARCH PAPER

Systems biology approaches to study the molecular effects of caloric restriction and polyphenols on aging processes

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Abstract Worldwide population is aging, and a large part of the growing burden associated with age-related conditions can be prevented or delayed by promoting healthy lifestyle and normalizing metabolic risk factors. However, a better understanding of the pleiotropic effects of available nutritional interventions and their influence on the multiple processes affected by aging is needed to select and implement the most promising actions. New methods of analysis are required to tackle the complexity of the interplay between nutritional interventions and aging, and to make sense of a growing amount of -omics data being produced for this purpose. In this paper, we review how various systems biology-inspired methods of analysis can be applied to the study of the molecular basis of nutritional interventions promoting healthy aging, notably caloric restriction and polyphenol supplementation. We specifically focus on the role that different versions of network analysis, molecular signature identification and multi-omics data integration are playing in elucidating the complex mechanisms underlying nutrition, and provide some examples on how to extend the application of these methods using available microarray data.

Keywords Network analysis · Multi-omics integration · Glutamate receptors · Oxidative stress · Inflammation · Healthy aging

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The molecular basis of nutritional strategies to promote healthy aging

Global life expectancy and proportion of people aged over 60 years are increasing. Aging-associated comorbidities will become the next global public health challenge in the context of westernization of daily habits and rising prevalence of risk factors for non-communicable chronic diseases. This situation is thus calling for prophylactic strategies to promote healthy aging (Suzman et al. 2015).

Aging is characterized by a progressive appearance of various dysfunctions that lead to physical and metabolic frailty, which increase the risk of developing various diseases (Lee et al. 2011). Notably, antioxidant and anti-inflammatory capacity are affected with increasing age as is the release and accumulation of stressors which result in a state of chronic low-grade inflammation and oxidative stress conceptualized, respectively, as "inflamm-aging" and "the free radical theory of aging" (Rubinsztein et al. 2011; Baylis et al. 2013; Beekman et al. 2013; Cevenini et al. 2013; Castellani et al. 2015). Another hallmark of aging is the progressive decline of the autophagy process, which normally plays a protective, anti-cytotoxic role by degrading protein aggregates and dysfunctional cellular components including mitochondria (Rubinsztein et al. 2011). By doing so, autophagy prevents the release of pro-oxidative and pro-inflammatory toxins, but also regulates

inflammation by exerting a control over the NLRP3 inflammasome and interleukin 1β production (Rubinsztein et al. 2011). Protein synthesis rates are also decreased in the aging individuals, which, when combined with the often-inadequate physical activity and nutritional habits of this population, increases risk of sarcopenia with ensuing metabolic dysfunctions (Beyer et al. 2012; Fabian et al. 2012).

Age-related dysfunctions thus arise from several molecular systems that share central molecular effectors particularly in the sirtuin family (SIRTs, regulators of cellular energy metabolism and mitochondrial biogenesis) and adenosine monophosphate-activated protein kinase (AMPK, central regulator of energy metabolism) and their downstream targets (Salminen and Kaarniranta 2012; Merksamer et al. 2013). Dysfunctions in those systems principally result in decreased capacity to respond to stressors, to regulate mitochondrial function and energy metabolism and, through their interaction with the mammalian target of rapamycin (mTOR), to impaired autophagy mechanisms (Rubinsztein et al. 2011; Salminen and Kaarniranta 2012; Merksamer et al. 2013).

Altogether, these alterations affect energy homeostasis and increase the risk of pathologies that share common inflammatory and oxidative basis such as obesity, insulin resistance, type II diabetes and cardiovascular diseases (Ceriello et al. 2004; Scrivo et al. 2011; North and Sinclair 2012). In the aging brain, the excessive release of oxidative and inflammatory mediators coupled with impaired autophagy mechanisms result in microvascular dysfunctions, protein oxidation, lipid peroxidation and DNA damage, which eventually lead to ischemic damages, abnormal protein aggregation, neuronal inflammation and cell death common to a number of neurodegenerative disorders (Rubinsztein et al. 2011; Rege et al. 2014; Salminen and Paul 2014). Moreover, excessive oxidative stress alters synaptic transmission and neuronal excitability, which leads to structural damage of the central nervous system (CNS) and decline of cognitive functions (Rizzo et al. 2014; Salminen and Paul 2014).

Caloric restriction

The pathophysiological implications of aging are thus broad and include multiple systems. Promotion of healthy aging should therefore similarly have multiple pleiotropic targets of action. Approaches aimed at improving lifestyle and dietary habits are part of those strategies that have large spectrum or action. Caloric restriction (CR), referring to 20–40 % reduction in habitual daily energy intake, is one such intervention that has largely been investigated since it was first identified to have potentially life-increasing benefits in yeast and, more recently, in mammalian models (Speakman and Mitchell 2011). It was postulated that CR benefits result from evolutionary mechanisms that halts cell division and energy-requiring functions under conditions of energy imbalance (Speakman and Mitchell 2011). The benefits of CR on slowing or preventing age-related dysfunctions are the result of its influence on the many systemic effectors previously mentioned as being affected in aging. This is demonstrated by several studies which showed that CR (1) negatively regulates the insulin-like growth factor (IGF-1)/insulin receptor substrate (IRS)/PI3k–Atk pathways, (2) activates SIRT family members, (3) activates AMPK (through decreased ATP/AMP ratio), (4) inhibits the mTOR pathway, (5) improves fatty acid metabolism (via CPT-1 and SREBP-1), (6) decreases the release of ROS and pro-inflammatory compounds and (7) modulates the expression of genes implicated in neuroprotection (Lee et al. 2000; Rubinsztein et al. 2011; Speakman and Mitchell 2011; Dai et al. 2014).

These effectors have been shown to have organ-specific effects. They partially act in synergy to improve muscle energy metabolism, prevent muscle loss and mitochondrial dysfunction in mice (Jang et al. 2012; Lin et al. 2014; Chen et al. 2015) and induce mitochondrial biogenesis in the skeletal muscle (of human as well), the heart, the adipose tissue and the brain (Baur 2010). CR also activates autophagy (Rubinsztein et al. 2011; Speakman and Mitchell 2011). In the aging rat brain, CR improved ketone body metabolism and blood flow, often impaired in neurodegenerative disorders (Lin et al. 2015). CR also delayed neurodegeneration in aging mice through SIRT1 (Graff et al. 2013), improved learning through PI3k/Akt pathway (Ma et al. 2014) and was demonstrated to attenuate amyloid β neuropathology in aged mice with Alzheimer's disease (Wang et al. 2005). Finally, one of the few investigation conducted in humans showed that a 3-month CR intervention improved memory in the elderly (Witte et al. 2009).

Calorie restriction mimetics: the case of polyphenols

Caloric restriction, however, benefits from being initiated early in life to yield the most positive effects on aging, particularly neuroprotective effects and was rarely investigated in human partly because it is often accompanied by side effects that make it hardly sustainable in the long-term (Speakman and Mitchell 2011). Alternative interventions that would mimic—at least partially—the molecular and physiological benefits of CR are thus of interest (Speakman and Mitchell 2011). Intermittent CR (or intermittent fasting) is one such alternative previously shown to decrease oxidative stress levels and improve insulin sensitivity in humans, but more research has yet to be done in this area

(Wegman et al. 2015). Polyphenols—a class of antioxidant phytochemicals mostly found in plant-derived produce such as fruits and vegetables (and their derivatives such as juices and wines), nuts, spices and grains (tea and chocolate)—represent another nutritional alternative to CR.

In rats, resveratrol supplementation prevented age-induced muscle loss (Jang et al. 2012; Joseph et al. 2013), suppressed pro-apoptotic signaling in senescent heart and prevented age-related heart dysfunction (Sin et al. 2014). Moreover, supplementation with polyphenol-rich blueberries increased IGF-1 expression in hippocampus of aged rats, improving hippocampal plasticity and memory performances (Casadesus et al. 2004). In addition, resveratrol supplementation in non-human primates increased working (as did CR) and spatial memory performance (Dal-Pan et al. 2011). Furthermore, this class of compounds was shown to mimic the effects of CR at the transcriptomic levels notably in reducing signs of aging (Barger et al. 2008; Pearson et al. 2008; Vidal et al. 2011). In fact, polyphenols and resveratrol mediate multiple of the previously described pathways such as SIRTs, AMPK and mTOR (Lam et al. 2013). In longitudinal clinical interventions conducted in humans, increased consumption of polyphenol-rich and Mediterranean-type food items such as extra-virgin olive oil and nuts has been associated with markers of healthy aging such as improvement of cardiovascular risk factors and cognition (Valls-Pedret et al. 2012; Martinez-Lapiscina et al. 2013; Zhu et al. 2014; Houston et al. 2015). Moreover, a resveratrol supplementation study in healthy aged individuals improved brain functional connectivity, which correlated with improved glucose metabolism and memory (Witte et al. 2014).

Challenges facing novel -omics technologies

Nutritional interventions aimed at promoting healthy aging influence multiple systems and have broad, and probably largely unknown, range of effectors. Furthermore, most investigations were conducted in animal models or in aged cohorts in whom the optimal intervention window to obtain significant life span improvements might be passed. On the other hand, longitudinal studies measuring hard endpoints such as decreases in aging-related morbidities or life span extension would require large investment of both time and capital. Understanding of the mechanisms associated with healthy aging promoting interventions and identifying potent biomarkers able to monitor the early efficacy of those interventions in younger cohorts are thus needed (Belsky et al. 2015). Moreover, interindividual variability in response to interventions adds to the necessity of identifying biomarkers of responders (Barberger-Gateau 2014).

Luckily, those needs can be fulfilled by recent high-throughput -omics capabilities and integrative systems biology approaches (Corthesy-Theulaz et al. 2005). These methods offer novel approaches to understanding the complex molecular mechanisms by which nutrition affects—patho—physiological processes (e.g., aging) in this increasingly data-rich field. Nutritional research has in fact gathered knowledge on the interaction of nutrients/nutritional habits with an individual's genome or between gene polymorphisms and individual responses to nutrients/nutritional habits. Moreover, data on how nutrition affects DNA methylation (nutritional epigenomics), gene expression (transcriptomics) and protein expression or post-translational modifications (proteomics), and how these changes affect a large number of metabolites (metabolomics) continue to be produced. As a final layer of complexity, gut microbiota composition (metagenomics) is increasingly recognized as crucial in modulating the two-way relationship between nutrition and host phenotypes.

The recent, and ever-growing, availability of -omics data will widen the window of observation of biological and pathophysiological processes that often involve multiple systems from different cell and/or tissue types. To fully profit from these advances, however, new methodologies will need to be developed and deepened in order to successfully integrate the information provided by the different levels of -omics into comprehensive models in order to avoid the risk of drowning under the over-abundance of such data.

The following sections provide an overview of such integrative approaches and will include an example on how these methods can be used to uncover new knowledge from existing microarray data produced to investigate the molecular impact of CR and resveratrol supplementation on brain tissues of young and aged mice.

Systems biology

Integrative approaches to studying biological systems are often the focus of the emerging field of systems biology. While a definitive and agreed-upon definition of what constitutes a systems biology method is still missing, it is generally accepted that the final objective is the study of complex biological systems using a holistic approach and relying on system-wide knowledge and high-throughput data. Examples of the methods employed are network-based analysis, methods based on molecular signatures, multi-omics data integration and system-wide modeling. Herein, we will briefly review how the first three concepts have been applied to the discovery of the connections between nutrition and healthy aging. A specific case study will be presented as an illustrative example, which includes a novel type of molecular signature combined with network analysis. We will not cover other important areas of

systems biology that would be best reviewed separately, such as the field of multi-scale modeling of biological systems, that also play a prominent role in the research on aging (see for example Milanesi et al. 2009).

Network-based analysis

Complex molecular systems such as gene regulation, protein–protein interactions (PPI) or the metabolic machinery, can be conveniently represented as networks. In such a network representation, molecules are shown as nodes, and the relationship between molecules as edges. Relationships can be correlations between gene expression levels, PPIs, and metabolic interactions coming from experimental studies (and derived from statistical associations) or databases, or curated from the literature.

The study of biological networks and their modeling, analysis and visualization represent powerful and flexible tools that can be useful in gathering data produced by high-throughput techniques and in helping uncover the knowledge thus generated. Information used to build networks can be qualitative or quantitative (unweighted vs weighted interactions), causal or correlative (directed vs undirected edges), qualified according to strength of evidence (interactions with qualifying labels such as "experimental," "literature," "indirect," "correlation-based"). Furthermore, such networks can be curated to specific cell type, tissue or condition by, for example, only considering genes or proteins ubiquitously expressed/present in all tissues and in the tissue of interest (Tegner et al. 2007).

Two approaches can be used to analyze biological networks, namely the seeded and genome-wide approaches (Parikshak et al. 2015). The seeded approach uses biomolecules of interest (e.g., obtained from experimental or genetic analysis) typically in the form of a set of differentially expressed genes or proteins, as the basis to build a network by addition of highly connected neighbors of those seeds. Upon optional network module detection, functional annotation analysis can then be carried out using seeds and their highly connected neighbors as input. This method as the advantage of adding information missed by the scarcity of biomolecules experimentally quantified and can potentially increase the significance of the results of a traditional functional analysis such as GO enrichment analysis.

The genome-wide approach is a slightly more advanced version in that it identifies modules of highly connected nodes within a comprehensive genome-wide network. Biomolecules of interest are then mapped onto the genome-wide network, and modules significantly enriched with biomolecules of interest are identified and analyzed for functional enrichment. The advantage of this approach is that modules in molecular networks have been shown to closely overlap with known biological functions (Ravasz et al. 2002; Parikshak et al. 2015), thus increasing the effectiveness of the functional analysis step.

A limited number of studies in nutritional research have used systems biology approaches. For example, seeded approach to network analysis was applied by Wuttke et al. (2012). The authors started with a list of CR-related genes (used as seeds) compiled from the literature and expanded it by addition of their highly interacting neighbors in order to predict novel CR-influenced genes in different organisms. Using the expanded gene list, they then performed a large-scale comparison of datasets (interactomes and transcriptomes) from multiple organisms. This enabled them to identify the most essential elements as those most conserved between organisms and conclude that life span extension operates via ancient rejuvenation process derived from gametogenesis.

The work of Morine et al. is an example of genome-wide approach to network analysis in which the authors studied the transcriptomic signature of adipose tissue of obese IL-1RI−/− mice subjected to high-fat feeding (Morine et al. 2013). Following intervention, markers of insulin sensitivity and inflammation in adipose tissue were assessed with the aid of a PPI/regulatory network of innate immunity and identified a significantly enriched module highlighting potentially novel inflammatory mediators of adipogenesis.

These studies demonstrated how network analysis can clarify the multi-systemic impacts of nutrition and how it could be applied to further the understanding of the mechanisms underlying healthy aging promoting interventions.

Molecular signatures

Another powerful tool for the study of -omics data is the concept of molecular signatures. Network analysis and signature analysis represent complementary approaches to the study of complex systems. While network analysis seeks to capture and manage the complexity of a biological system, signature analysis summarizes the essential features of such a system in the most possible succinct way. The simplest form of molecular signature is a list of biomolecules (genes, proteins or metabolites) carefully selected for their discriminating value; such signatures have long been studied for their potential as diagnostic biomarkers. Since our understanding of the effects of nutrition on aging in humans would greatly benefit from an effective form of monitoring, the need for biomarkers in this area of research is a pressing one. Traditional biomarker identification identified a set of genes rapidly affected upon initiation of CR in old mice (Dhahbi et al.

2004). Those genes could thus be good candidate biomarkers to rapidly assess the efficacy of, and detect responders from non-responders to CR.

Enhanced network-based analysis: combining networks and signatures

Besides their use as biomarkers, molecular signatures can also be used as a selection tool to increase effectiveness of network analysis. Network analysis and signature extraction can be combined in a single workflow in at least two possible ways, which we term the "summary" and the "feature-list" approaches. In the *summary* approach, the signature extraction operation follows the network analysis and is performed on the results of the latter. In this case, signatures are typically used as a succinct way to summarize the complex outcome of the network analysis as a way to facilitate, for example, the comparison of results across treatments, time points, tissues or conditions. The advantage of the summary approach is that it results in a list of biomolecules that are more relevant to the question of interest than a signature obtained directly from the -omics data, because of the discriminating power of the intervening network analysis (Kelder et al. 2014). A summary approach was selected to study the hepatic molecular network underlying type 2 diabetes mellitus using transcriptional data obtained from experiments with different drug and dietary treatments (Kelder et al. 2014). After performing a genome-wide network analysis, the authors compared signatures of the different interventions, which highlighted functionally related nodes that may be used as target to future anti-diabetic treatments or as biomarkers for determining their efficacy (Fig. 1).

In the *feature-list* approach, all -omics data are used as input for the signature extraction operation, and the biomolecules included in the signatures are then used as input to network analysis (e.g., as the seeds in case of the seeded approach). The advantage of this approach is that the ensuing network analysis inherits all the potential benefits of the selected signature extraction algorithm, possibly overcoming the known limitations of traditional statistical methods for the identification of differently expressed genes.

As a demonstration of the feature-list approach, we apply our recently developed signature identification method followed by network analysis, in order to study the role of CR and resveratrol supplementation on brain aging in mice (Fig. 2). The novelty represented by the use of a transcriptional signature enabled a more sensitive analysis than the one resulting from a list of differentially expressed genes obtained with traditional methods. Other benefits afforded by our signature identification method are robustness to batch effects and to differences in processing methods, and the possibility of using expression data from multiple sources, none of which were relevant for this particular study. We re-analyzed the microarray data produced by Barger et al. (2008) on the role of CR and resveratrol at low doses on brain aging. Briefly, 14-month-old male mice fed a control diet were randomly assigned to one of the following: a CR (63 kcal weekly) diet, a resveratrol-supplemented (50 mg resveratrol per kilogram of body weight) control diet and a control diet. Brain tissues were collected at 30 month, while brain tissues from 5-month-old mice fed the control diet were also collected and served as young controls. Identification of the transcriptional signature was done using an enhanced version of a rank-based classification method described in the Supplementary Material (Lauria 2013; Tarca et al. 2013; Lauria et al. 2015).

Following a version of the seeded approach to network analysis, a network was then build using the signature genes as seeds with the NetWalker tool (Komurov et al. 2012) in combination with the BioGrid PPI network for *mus musculus* (rel. 3.2.121). The resulting *NetWalker network*, thus, contained a subset of highly connected signature genes (i.e., those interacting above a determined threshold), augmented by additional genes that were connected to the signature ones through above-threshold interactions. The genes obtained from such network enrichment analysis were then used to extract the most representative GO biological process terms (i.e., the ones that are over-represented, but that do not refer to most general biological processes). Pathway analysis was performed using DAVID web-based tool using hypergeometric distribution test and a FDR-adjusted p value threshold of 0.05 (Huang da et al. 2009).

Using this method, we identified transcriptomic signatures and related networks for age (5- vs 30-month-old mice), CR (30 month control vs 30 month CR) and resveratrol (30 month control vs 30 month resveratrol).

In the brain aging process (young vs old mice on control diet), the analysis of the Netwalker-enriched network (see Fig. 2) highlighted biological functions associated with immune processes and inflammation, steroid hormone receptor signaling, neurogenesis and histone modification (Supplementary Table 1). Caloric restriction and resveratrol supplementation produced overlapping results related to neuronal functions including regulation of synaptic transmission and glutamate receptor signaling. In addition, GO BP term associated with response to oxidative stress was significantly enriched in resveratrol-treated mice and with a trend toward significance in CR-exposed mice, while inflammatory processes were exclusively associated with resveratrol supplementation. Overlapping GO BP terms between both dietary interventions demonstrate an

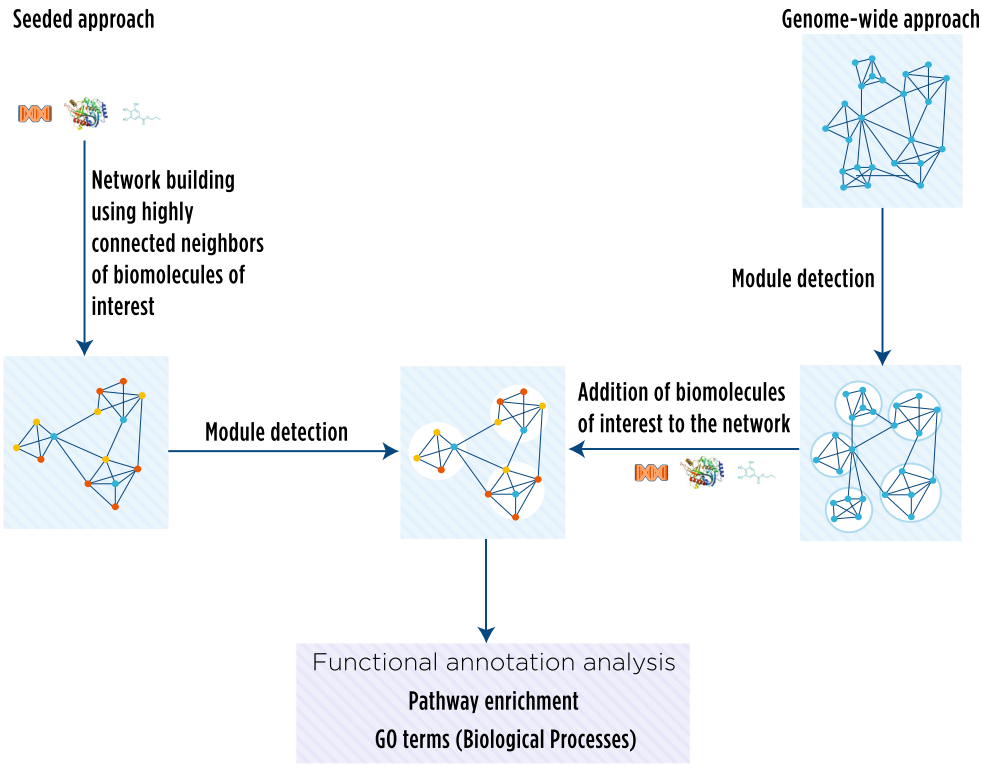

Fig. 1 Illustration of the two main approaches to network analysis, namely the seeded (*left*) and genome-wide (*right*)

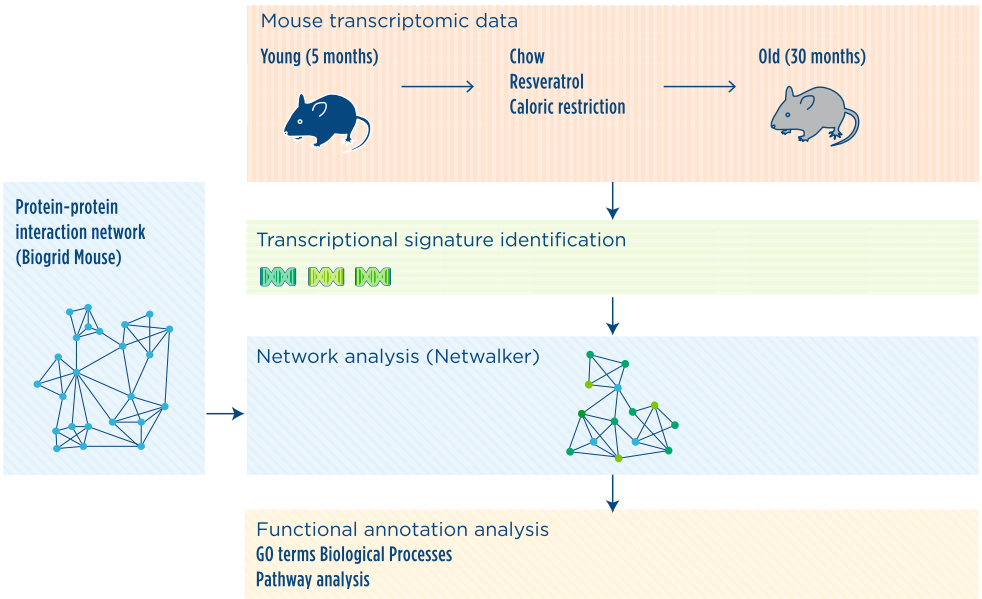

Fig. 2 Schematic representation of the case study workflow. Transcriptomic data from brain tissue of treatment groups (caloric restriction, low-dose resveratrol supplementation and control diet) from young and aged mice were analyzed using a novel approach enabling the identification of transcriptional signature characterizing each group. Network analysis was then performed using Netwalker method and the mouse-specific BioGRID PPI database. Finally, the functional enrichments of the genes obtained from the network analysis were characterized by investigating over-represented gene ontology biological processes and pathways

effect on synaptic transmission particularly associated with ionotropic glutamate receptors, while only resveratrol supplementation had a significant effect on NF-kB Signaling (Supplementary Table 1).

Complete lists of Materials and methods and results are available in the supplementary material.

Although these findings largely confirm those of Barger et al. and are in agreement with previous knowledge (Lam

et al. 2013; Marchal et al. 2013), our method was also able to uncover the effect of both interventions on unrecognized molecular processes such as those related to synaptic transmission and glutamatergic signaling. In particular, they suggest the involvement of glutamatergic ionotropic receptors, including AMPA receptor subunits 1, 2 and 3 (GRIA1, 2 and 3), a result reported in connection with CR in rats (Shi et al. 2007) and very recently confirmed also in mice (Schafer et al. 2015). The involvement of these key signaling molecules for brain functions reinforces the notion that dietary interventions such as those studied herein hold the ability to modulate brain health and function. This is of particular interest for resveratrol supplementation as knowledge was limited on its ability to modulate glutamatergic transmission and further supports the investigation of resveratrol in the treatment of various aging-related central nervous system disorders. Interestingly, this resveratrol effect has been confirmed independently in rats (Wang et al. 2015).

Moreover, our analyses confirm the effects of age on NF-kB signaling and inflammation, particularly on Toll-like receptor (TLR) signaling, but also interestingly highlight impacts of aging on the regulation of neurogenesis, known to decline with advancing age (Kuhn et al. 1996; Villeda et al. 2011). Among the age-related plasma factors correlated strongly with decreased neurogenesis, β2 microglobulin was found also in our age signature and in the Netwalker-enriched network, suggesting that immunological response could be related to age-related impairments of neurogenesis (Villeda et al. 2011).

This re-examination of previously published data shows that a novel transcriptional signature identification combined with carefully matched network analysis can provide new insight into the mechanisms involved in the complex interaction of nutritional interventions and aging and guide potential new therapeutic approaches.

Multi-omics data integration

Traditional and network-based analysis of the major platforms of comprehensive -omics can be used to study the influence of nutritional interventions (for instance CR and polyphenol supplementation) on biological processes such as those involved in aging. However, previously stated investigations assessed biological processes/dysfunctions on the basis of data representative of single-omics level. Systems biology would benefit from a holistic system-wide approach by integrating different -omics levels with the following methodologies and tools.

Many approaches to perform multi-omics data integration have been developed and have different usefulness depending on the biological question to be addressed and the -omics data available. For example, -omics integration can be used to understand the genetic architecture of complex traits by integrating genomics with transcriptomics, proteomics, and/or metabolomics, to evaluate the role of genetic variations in intermediate molecular phenotype of a complex trait. Such studies are called expression QTL (eQTL; Schadt 2006), proteomics QTL (pQTL) or metabolomics QTL (mQTL) analysis depending on the data available. Multi-omics analysis can also be used to obtain a comprehensive modeling of -omics profiles to be used as biomarker of disease, disease state and/or treatment efficacy, or to determine key elements, which correlate with a specific phenotype, or for exploring host–microbiota interactions.

There are two main strategies for integration: multistage (stepwise or sequential) and meta-dimensional (simultaneous) analyses (Ritchie et al. 2015). The multistage analyses are stepwise or hierarchical methods that rely on the central dogma of biology in which variations at the DNA level will hierarchically affect RNA levels, protein expression and so on. These methods reduce the search space through different steps of analysis but are challenged by the accumulating evidence (e.g., notably epigenetic modifications, microbiota–host interactions) that question the linearity of such dogma and thus the ability of this model to capture every inter-omics interaction.

The more computationally demanding meta-dimensional analyses simultaneously combine multi-omics data to produce complex models defined by multiple differently scaled variables. Those approaches are of interest since every potential relationship between different -omics levels are considered and, thus, it captures potential inter-omics interactions more realistically.

Multi-omics integration can be achieved simply by separately analyzing different -omic data and then combining the obtained results together in order to draw global conclusions. By doing so, this methodology does not, however, provide statistics on the interactions between -omics levels since they were treated separately. Statistical integration methods perform statistical association between biomolecules from different -omics levels (Cavill et al. 2015; Ritchie et al. 2015). The main statistical integration approaches can either be based on correlation, concatenation, multivariate or pathway analysis, which are further detailed in Table 1 along with suggestions of useful tools that implement each approach.

To our knowledge, there are no examples of multi-omics integration analysis applied in the field of CR or polyphenol supplementation on age-related pathologies. Although relatively sparse, there are nonetheless some examples of studies that used those approaches to analyze the effect of dietary interventions on other phenotypes. For instance, Montastier and collaborators have recently

Table 1 Details of the four main statistical integration approaches and suggested tools

Method	Comments	Suggested tools	Tool ref.
Correlation-based integration	Seeks to identify correlative links between elements of different -omics datasets	3Omics	Kuo et al. (2013)
Concatenation-based integration	Groups different -omics or single-omic dataset from different experiments or experimental conditions into a single matrix and then performs integrated analysis	Multiple co-inertia analysis (MCIA)	Meng et al. (2014)
		Joint and Individual Variation Explained (JIVE)	Lock et al. (2013)
Multivariate-based integration	Relies on multivariate statistical methods such as canonical correlation analysis (CCA) (Jozefczuk et al. 2010) and orthogonal partial least squares discriminant analysis (OPLS-DA) (Boccard and Rutledge 2013) to calculate relationship between different levels of -omics data	R package mixOmics	González et al. (2011)
Pathway-based integration	Integrates different -omics levels by relying on existing biological knowledge gathered from metabolic pathways such as Kegg and wikipathways (Kutmon et al. 2015)	InCroMAP and IMPALA for integrated pathway-based analysis	Eichner et al. (2014), Kamburov et al. (2011)
		SAMNetWeb to generate biological networks with transcriptomics and proteomics data	Gosline et al. (2015)
		MetScape cytoscape plugin to produce metabolic networks from transcriptomics and metabolite data	Karnovsky et al. (2012)

performed a multistage analysis using both single and multi-omics networks to show metabolic alterations occurring during weight change in response to CR (Montastier et al. 2015). To do so, they first inferred a partial correlation network for each -omics level and then constructed multi-omics networks linking each pairs of data type using regularized canonical correlation analysis, which successfully infers a gene/phenotype network (Rengel et al. 2012). They then merged single and multi-omics networks together and performed module detection analyses. Temporal analysis of the modules successfully revealed both shared and time-specific biological signatures in response to metabolic variations occurring during weight changes.

Finally, multi-omics integration that would take into account cell or tissue types, as proposed by Tegner and collaborators, would be desirable to investigate the cell- or tissue-specific effects of pleiotropic interventions such as nutritional—and lifestyle—modification modulating progression of complex, multi-system diseases (Tegner et al. 2007).

Conclusion

There is a pressing need for new methods to tackle the complexity of the interplay between nutritional interventions and aging and to make sense of a growing amount of -omics data being produced for this purpose. In this review, we detailed systems biology-inspired methods of analysis that could fulfill this need and be applied to the burgeoning field of nutrition. This review has illustrated the potential of network analysis, molecular signature identification and multi-omics data integration to generate candidate biomarkers and novel molecular mechanisms in an unbiased fashion. We foresee that the real potential for nutritional systems biology applications will lie in multi-knowledge integration strategies that will, by including information at different levels, shed light on gene–nutrient interactions with a degree of accuracy and completeness not yet achievable today.

References

Barberger-Gateau P (2014) Nutrition and brain aging: how can we move ahead? Eur J Clin Nutr 68:1245–1249

Barger JL, Kayo T et al (2008) A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. PLoS ONE 3:e2264

Baur JA (2010) Resveratrol, sirtuins, and the promise of a DR mimetic. Mech Ageing Dev 131:261–269

Baylis D, Bartlett DB et al (2013) Understanding how we age: insights into inflammaging. Longev Healthspan 2:8

Beekman M, Blanche H et al (2013) Genome-wide linkage analysis for human longevity: Genetics of Healthy Aging Study. Aging Cell 12:184–193

Belsky DW, Caspi A et al (2015) Quantification of biological aging in young adults. Proc Natl Acad Sci USA 112(30):E4104–E4110. doi:10.1073/pnas.1506264112

Beyer I, Mets T et al (2012) Chronic low-grade inflammation and age-related sarcopenia. Curr Opin Clin Nutr Metab Care 15:12–22

Boccard J, Rutledge DN (2013) A consensus orthogonal partial least squares discriminant analysis (OPLS-DA) strategy for multi-block Omics data fusion. Anal Chim Acta 769:30–39

Casadesus G, Shukitt-Hale B et al (2004) Modulation of hippocampal plasticity and cognitive behavior by short-term blueberry supplementation in aged rats. Nutr Neurosci 7:309–316
Castellani GC, Menichetti G et al (2015) Systems medicine of inflammaging. Brief Bioinform 1–14. doi:10.1093/bib/bbv062
Cavill R, Jennen D et al (2015) Transcriptomic and metabolomic data integration. Brief Bioinform 1–11. doi:10.1093/bib/bbv090
Ceriello A, Motz E (2004) Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 24:816–823
Cevenini E, Monti D et al (2013) Inflamm-ageing. Curr Opin Clin Nutr Metab Care 16:14–20
Chen CN, Lin SY et al (2015) Late-onset caloric restriction alters skeletal muscle metabolism by modulating pyruvate metabolism. Am J Physiol Endocrinol Metab 308:E942–E949
Corthesy-Theulaz I, den Dunnen JT et al (2005) Nutrigenomics: the impact of biomics technology on nutrition research. Ann Nutr Metab 49:355–365
da Huang W, Sherman BT et al (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37:1–13
Dai DF, Karunadharma PP et al (2014) Altered proteome turnover and remodeling by short-term caloric restriction or rapamycin rejuvenate the aging heart. Aging Cell 13:529–539
Dal-Pan A, Pifferi F et al (2011) Cognitive performances are selectively enhanced during chronic caloric restriction or resveratrol supplementation in a primate. PLoS ONE 6:e16581
Dhahbi JM, Kim HJ et al (2004) Temporal linkage between the phenotypic and genomic responses to caloric restriction. Proc Natl Acad Sci USA 101:5524–5529
Eichner J, Rosenbaum L et al (2014) Integrated enrichment analysis and pathway-centered visualization of metabolomics, proteomics, transcriptomics, and genomics data by using the InCroMAP software. J Chromatogr B Analyt Technol Biomed Life Sci 966:77–82
Fabian E, Bogner M et al (2012) Vitamin status in elderly people in relation to the use of nutritional supplements. J Nutr Health Aging 16:206–212
González I, Lé Cao K et al (2011) mixOmics: Omics Data Integration Project. http://www.mixomics.org
Gosline SJ, Oh C et al (2015) SAMNetWeb: identifying condition-specific networks linking signaling and transcription. Bioinformatics 31:1124–1126
Graff J, Kahn M et al (2013) A dietary regimen of caloric restriction or pharmacological activation of SIRT1 to delay the onset of neurodegeneration. J Neurosci 33:8951–8960
Houston DK, Leng X et al (2015) A long-term intensive lifestyle intervention and physical function: the look AHEAD Movement and Memory Study. Obesity (Silver Spring) 23:77–84
Jang YC, Liu Y et al (2012) Dietary restriction attenuates age-associated muscle atrophy by lowering oxidative stress in mice even in complete absence of CuZnSOD. Aging Cell 11:770–782
Joseph AM, Malamo AG et al (2013) Short-term caloric restriction, resveratrol, or combined treatment regimens initiated in late-life alter mitochondrial protein expression profiles in a fiber-type specific manner in aged animals. Exp Gerontol 48:858–868
Jozefczuk S, Klie S et al (2010) Metabolomic and transcriptomic stress response of *Escherichia coli*. Mol Syst Biol 6:364
Kamburov A, Cavill R et al (2011) Integrated pathway-level analysis of transcriptomics and metabolomics data with IMPaLA. Bioinformatics 27:2917–2918
Karnovsky A, Weymouth T et al (2012) Metscape 2 bioinformatics tool for the analysis and visualization of metabolomics and gene expression data. Bioinformatics 28:373–380
Kelder T, Verschuren L et al (2014) Network signatures link hepatic effects of anti-diabetic interventions with systemic disease parameters. BMC Syst Biol 8:108
Komurov K, Dursun S et al (2012) NetWalker: a contextual network analysis tool for functional genomics. BMC Genom 13:282
Kuhn HG, Dickinson-Anson H et al (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci 16:2027–2033
Kuo TC, Tian TF et al (2013) 3Omics: a web-based systems biology tool for analysis, integration and visualization of human transcriptomic, proteomic and metabolomic data. BMC Syst Biol 7:64
Kutmon M, Riutta A et al (2015) WikiPathways: capturing the full diversity of pathway knowledge. Nucleic Acids Res. doi:10.1093/nar/gkv1024
Lam YY, Peterson CM et al (2013) Resveratrol vs. calorie restriction: data from rodents to humans. Exp Gerontol 48:1018–1024
Lauria M (2013) Rank-based transcriptional signatures: a novel approach to diagnostic biomarker definition and analysis. Syst Biomed 1:228–239
Lauria M, Moyseos P et al (2015) SCUDO: a tool for signature-based clustering of expression profiles. Nucleic Acids Res 43:W188–W192
Lee CK, Weindruch R et al (2000) Gene-expression profile of the ageing brain in mice. Nat Genet 25:294–297
Lee JS, Auyeung TW et al (2011) Physical frailty in older adults is associated with metabolic and atherosclerotic risk factors and cognitive impairment independent of muscle mass. J Nutr Health Aging 15:857–862
Lin AL, Coman D et al (2014) Caloric restriction impedes age-related decline of mitochondrial function and neuronal activity. J Cereb Blood Flow Metab 34:1440–1443
Lin AL, Zhang W et al (2015) Caloric restriction increases ketone bodies metabolism and preserves blood flow in aging brain. Neurobiol Aging 36:2296–2303
Lock EF, Hoadley KA et al (2013) Joint and individual variation explained (Jive) for integrated analysis of multiple data types. Ann Appl Stat 7:523–542
Ma L, Zhao Z et al (2014) Caloric restriction can improve learning ability in C57/BL mice via regulation of the insulin-PI3K/Akt signaling pathway. Neurol Sci 35:1381–1386
Marchal J, Dal-Pan A et al (2013) Calorie restriction and resveratrol supplementation prevent age-related DNA and RNA oxidative damage in a non-human primate. Exp Gerontol 48:992–1000
Martinez-Lapiscina EH, Clavero P et al (2013) Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial. J Nutr Health Aging 17:544–552
Meng C, Kuster B et al (2014) A multivariate approach to the integration of multi-omics datasets. BMC Bioinformatics 15:162
Merksamer PI, Liu Y et al (2013) The sirtuins, oxidative stress and aging: an emerging link. Aging (Albany NY) 5:144–150
Milanesi L, Romano P et al (2009) Trends in modeling biomedical complex systems. BMC Bioinform 10(Suppl 12):I1
Montastier E, Villa-Vialaneix N et al (2015) System model network for adipose tissue signatures related to weight changes in response to calorie restriction and subsequent weight maintenance. PLoS Comput Biol 11:e1004047
Morine MJ, Toomey S et al (2013) Network analysis of adipose tissue gene expression highlights altered metabolic and regulatory transcriptomic activity in high-fat-diet-fed IL-1RI knockout mice. J Nutr Biochem 24:788–795
North BJ, Sinclair DA (2012) The intersection between aging and cardiovascular disease. Circ Res 110:1097–1108
Parikshak NN, Gandal MJ et al (2015) Systems biology and gene networks in neurodevelopmental and neurodegenerative disorders. Nat Rev Genet 16:441–458

Pearson KJ, Baur JA et al (2008) Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab 8:157–168

Ravasz E, Somera AL et al (2002) Hierarchical organization of modularity in metabolic networks. Science 297:1551–1555

Rege SD, Geetha T et al (2014) Neuroprotective effects of resveratrol in Alzheimer disease pathology. Front Aging Neurosci 6:218

Rengel D, Arribat S et al (2012) A gene-phenotype network based on genetic variability for drought responses reveals key physiological processes in controlled and natural environments. PLoS ONE 7:e45249

Ritchie MD, Holzinger ER et al (2015) Methods of integrating data to uncover genotype-phenotype interactions. Nat Rev Genet 16:85–97

Rizzo V, Richman J et al (2014) Dissecting mechanisms of brain aging by studying the intrinsic excitability of neurons. Front Aging Neurosci 6:337

Rubinsztein DC, Marino G et al (2011) Autophagy and aging. Cell 146:682–695

Salminen A, Kaarniranta K (2012) AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. Ageing Res Rev 11:230–241

Salminen LE, Paul RH (2014) Oxidative stress and genetic markers of suboptimal antioxidant defense in the aging brain: a theoretical review. Rev Neurosci 25:805–819

Schadt EE (2006) Novel integrative genomics strategies to identify genes for complex traits. Anim Genet 37(Suppl 1):18–23

Schafer MJ, Dolgalev I et al (2015) Calorie restriction suppresses age-dependent hippocampal transcriptional signatures. PLoS ONE 10:e0133923

Scrivo R, Vasile M et al (2011) Inflammation as "common soil" of the multifactorial diseases. Autoimmun Rev 10:369–374

Shi L, Adams MM et al (2007) Caloric restriction eliminates the aging-related decline in NMDA and AMPA receptor subunits in the rat hippocampus and induces homeostasis. Exp Neurol 206:70–79

Sin TK, Yu AP et al (2014) Modulating effect of SIRT1 activation induced by resveratrol on Foxo1-associated apoptotic signalling in senescent heart. J Physiol 592:2535–2548

Speakman JR, Mitchell SE (2011) Caloric restriction. Mol Aspects Med 32:159–221

Suzman R, Beard JR et al (2015) Health in an ageing world—what do we know? Lancet 385:484–486

Tarca AL, Lauria M et al (2013) Strengths and limitations of microarray-based phenotype prediction: lessons learned from the IMPROVER Diagnostic Signature Challenge. Bioinformatics 29:2892–2899

Tegner J, Skogsberg J et al (2007) Thematic review series: systems biology approaches to metabolic and cardiovascular disorders. Multi-organ whole-genome measurements and reverse engineering to uncover gene networks underlying complex traits. J Lipid Res 48:267–277

Valls-Pedret C, Lamuela-Raventos RM et al (2012) Polyphenol-rich foods in the Mediterranean diet are associated with better cognitive function in elderly subjects at high cardiovascular risk. J Alzheimers Dis 29:773–782

Vidal M, Cusick ME et al (2011) Interactome networks and human disease. Cell 144:986–998

Villeda SA, Luo J et al (2011) The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 477:90–94

Wang J, Ho L et al (2005) Caloric restriction attenuates beta-amyloid neuropathology in a mouse model of Alzheimer's disease. FASEB J 19:659–661

Wang G, Amato S et al (2015) Resveratrol up-regulates AMPA receptor expression via AMP-activated protein kinase-mediated protein translation. Neuropharmacology 95:144–153

Wegman MP, Guo MH et al (2015) Practicality of intermittent fasting in humans and its effect on oxidative stress and genes related to aging and metabolism. Rejuvenation Res 18:162–172

Witte AV, Fobker M et al (2009) Caloric restriction improves memory in elderly humans. Proc Natl Acad Sci USA 106:1255–1260

Witte AV, Kerti L et al (2014) Effects of resveratrol on memory performance, hippocampal functional connectivity, and glucose metabolism in healthy older adults. J Neurosci 34:7862–7870

Wuttke D, Connor R et al (2012) Dissecting the gene network of dietary restriction to identify evolutionarily conserved pathways and new functional genes. PLoS Genet 8:e1002834

Zhu N, Jacobs DR Jr et al (2014) Cardiorespiratory fitness and cognitive function in middle age: the CARDIA study. Neurology 82:1339–1346