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Functional Genomics in the Study of Mind-Body Therapies

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ABSTRACT

Background: Mind-body therapies (MBTs) are used throughout the world in treatment, disease prevention, and health promotion. However, the mechanisms by which MBTs exert their positive effects are not well understood. Investigations into MBTs using functional genomics have revolutionized the understanding of MBT mechanisms and their effects on human physiology.

Methods: We searched the literature for the effects of MBTs on functional genomics determinants using MEDLINE, supplemented by a manual search of additional journals and a reference list review.

Results: We reviewed 15 trials that measured global or targeted transcriptomic, epigenomic, or proteomic changes in peripheral blood. Sample sizes ranged from small pilot studies (n=2) to large trials (n=500). While the reliability of individual genes from trial to trial was often inconsistent, genes related to inflammatory response, particularly those involved in the nuclear factor-kappa B (NF-κB) pathway, were consistently downregulated across most studies.

Conclusion: In general, existing trials focusing on gene expression changes brought about by MBTs have revealed intriguing connections to the immune system through the NF-κB cascade, to telomere maintenance, and to apoptotic regulation. However, these findings are limited to a small number of trials and relatively small sample sizes. More rigorous randomized controlled trials of healthy subjects and specific disease states are warranted. Future research should investigate functional genomics areas both upstream and downstream of MBT-related gene expression changes—from epigenomics to proteomics and metabolomics.

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INTRODUCTION

Much of medicine focuses on the most recent, cutting-edge technologic breakthroughs such as the bionic pancreas[1] or targeted gene therapy for treatment-resistant cancers.[2] In striking contrast, an area of expanding interest within medicine is based on practices that are hundreds, if not thousands, of years old. Mind-body therapies (MBTs), also known as mind-body medicine, draw upon rich traditions, as noted by the National Center for Complementary and Alternative Medicine, to "focus on the interactions among the brain, mind, body, and behavior, and on the powerful ways in which emotional, mental, social, spiritual, and behavioral factors can directly affect health" (nccam.nih.gov). According to the 2007 National Health Interview Survey (NHIS),[3] nearly 1 in 5 Americans reported using MBT—a category encompassing meditation, yoga, tai chi, qigong, biofeedback, progressive muscle relaxation, guided imagery, hypnosis, and deep breathing exercises. The percentage of respondents reporting the use of MBTs increased between the 2002 and the 2007 NHIS by at least 1 percentage point each for yoga, deep breathing, and meditation. Moreover, US adults spent more than $4.1 billion per year on MBT.[4] Initial findings from the 2012 NHIS show a continuing increase in the use of yoga—8.4% of Americans practiced yoga in 2012,[5] up from 6.1% in 2007.[3]

Many MBTs, such as yoga, tai chi, and qigong, originate from long-standing spiritual traditions. Other MBTs, like hypnosis and progressive muscule relaxation, have more nascent histories. While MBTs vary, they share several key similarities, especially in health applications. First, MBTs involve regulation of the mind's attention processes to impact the body's physiology.[6] Attention can be focused on a single object or mental process as in some forms of meditation, or on dynamic body postures as in tai chi or yoga. Second, practice of MBTs usually precipitates parasympathetic activation, demonstrated by reduced blood pressure, heart rate, and oxygen consumption and increased vagal tone (heart rate variability). This physiologic effect, initially described as the relaxation response by Herbert Benson,[7] is distinct from other parasympathetic-predominant states such as sleep and has been hypothesized to be a shared physiology among MBTs. Finally, studies correlate MBTs with various neurobiological changes, including alterations in cortical thickness,[8,9] default mode connectivity,[10,11] and decreased insulin resistance.[12] While the interplay between biological, psychological, biochemical, and genetic effects is not yet well understood, Taylor et al have hypothesized that the observed cortical, neuroendocrine, and molecular outcomes of regular MBT practice may be connected through an executive homeostatic network that links calming mental processes to the maintenance of homeostasis in physiological systems.[13]

Because of their wide range of effects, MBTs have been studied in a variety of disease states. Evidence has shown the efficacy of MBTs in treating conditions such as cardiovascular disease, inflammatory disease[6], hypertension,[6,14] irritable bowel syndrome,[15] insomnia,[6,16] chronic pain,[17,18] depression,[19,20,21] and posttraumatic stress disorder (PTSD).[22] In aggregate, the effectiveness of MBT interventions is modest,[21,23] and the specific mechanisms of action of MBT are not well understood.

NEW TOOLS FOR ANCIENT PRACTICES

To uncover genomic determinants and molecular pathways involved in the changes brought about by MBT, researchers are turning to functional genomics, a developing field that encompasses genomics, epigenomics, transcriptomics, proteomics, and metabolomics. Since the completion of the first draft of the human genome in 2003, there has been exponential growth in understanding the genomic determinants of disease and identifying biomarkers for disease management. Through the measurement of thousands of genomic determinants, functional genomics technologies are able to generate hypotheses about disease processes and intervention effects at molecular levels. More recently, functional genomics has been applied to further our understanding of MBT. A 2013 review described 3 early trials,[24] but no review has tackled the full breadth of functional genomics approaches to MBT research.

Genomics targets an individual's entire complement of genetic material. This DNA blueprint is used to build proteins through the processes of transcription and translation (Figure 1). While an organism's DNA sequence remains essentially unchanged throughout its lifespan (with some exceptions—for example, the mutations that can cause a cell line to become cancerous), diverse processes control how the genes encoded by a cell's DNA sequence are expressed. The story of how genes switch on and off throughout time, from fractions of seconds to generations, is complicated. Work from 2012 and 2013 reveals this story to be even more complicated than once thought.[25,26]

A broad array of processes modulates gene expression; a subset of these processes is the group of molecular mechanisms known collectively as epigenetics. Epigenetics includes stable, long-term changes in gene expression through DNA methylation, histone modification, and chromatin remodeling, some of which offspring can inherit.[27,28] By definition, epigenetic mechanisms do not involve alteration of the DNA sequence (ie, do not involve DNA mutation). The sum of active epigenetic mechanisms in a given cell comprises the epigenome; these mechanisms effectively activate and deactivate sections of the genome, enabling or preventing their transcription into RNA and subsequent translation into proteins. Histone protein modifications change the way the DNA strand is wrapped around the nucleosome, thus altering which regions are accessible for transcription. While many histone modifications have been reported in the literature (eg, lysine acetylation, lysine methylation, and serine phosphorylation), the functional meaning of these changes is not well understood.[29] DNA methylation involves the addition of a methyl group to the cytosine in a cytosine-guanine sequence (CpG). High-precision technologies are now able to analyze the methylation status per base level. While the majority of these pairings are scattered throughout the genome, approximately 10% of human CpGs cluster in distinct groupings known as CpG islands. Methylation of these CpG islands is believed to be responsible for long-term heritable changes as well as the process of cell lineage differentiation.[30] However, epigenomic comparison across tissue types is challenging because methylation patterns vary among different cell types.

Transcriptomics, or gene expression profiling, is the quantification of messenger RNA (mRNA) in specific cell or tissue types. While the genome itself

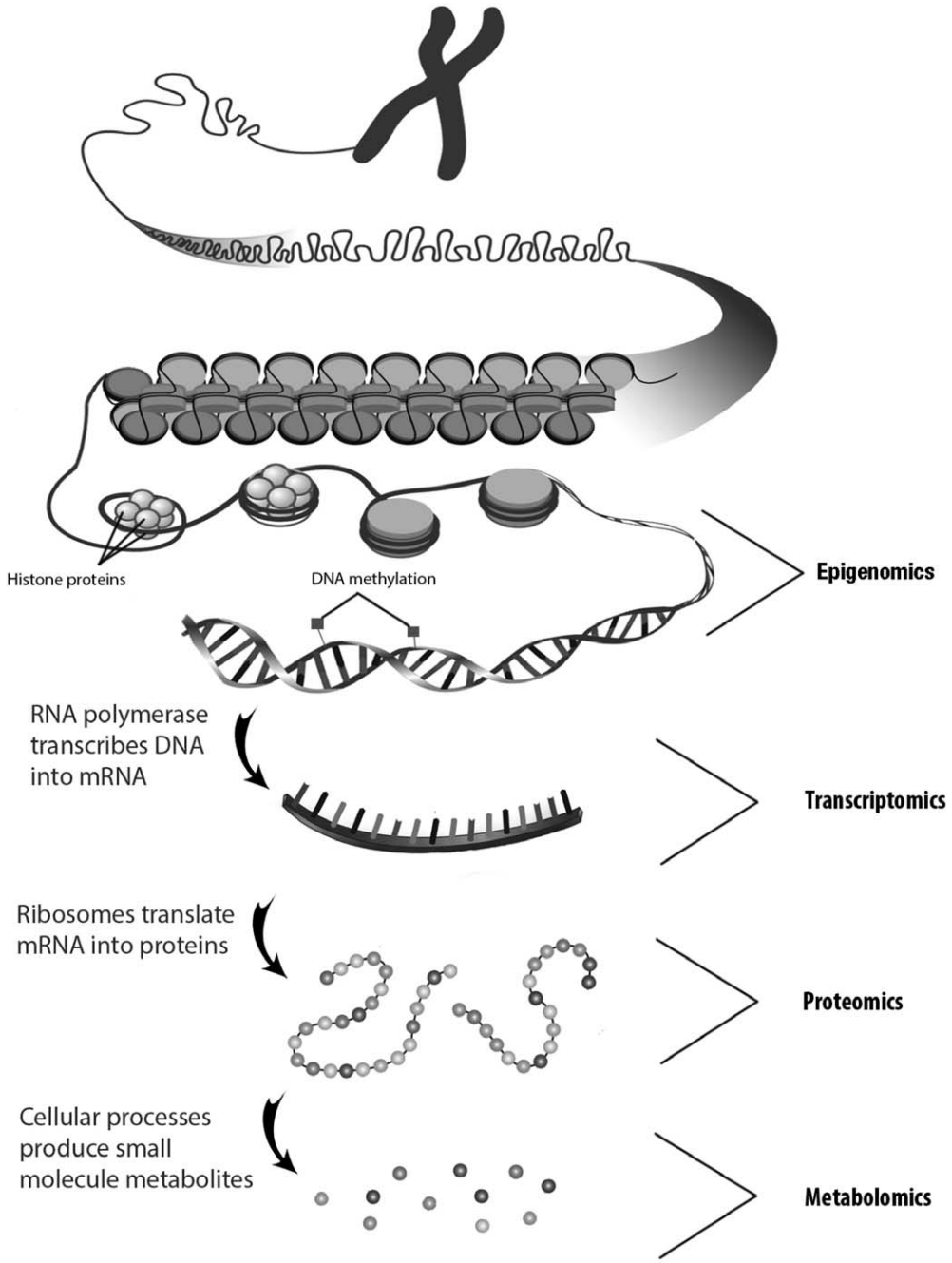

Figure 1. Functional genomics consists of several subfields, defined by their examination of different phases of the cellular cycle. Double helix DNA carries the genetic code, coiling around histone proteins within chromosomes. Epigenomics analyzes modification of histone proteins as well as methylation of regions rich in cytosine-guanine pairings on the DNA strand itself. Transcriptomics involves assessing gene expression by sequencing free-floating messenger RNA strands. Proteomics explores expression levels by identifying and quantifying amounts of circulating proteins. Metabolomics assesses small molecule end-products of cellular processes to quantify the rate at which various processes occur. (Source: Darryl Leja, of the National Human Genome Research Institute. www.genome.gov. Accessed September 23, 2014.)

is essentially stable for a given cell line, the transcriptome is constantly in flux, responding to external and internal conditions, and is thus a good indicator of gene activity and regulation. Several commonly used techniques for measuring gene expression activity are DNA microarrays, multigene transcriptional profiling, and next-generation sequencing technologies.[31] DNA microarrays allow for measurement of tens of thousands of genes in a single assay and are useful for generating hypotheses about intervention mechanisms and for understanding the pathophysiology of diseases, including the gene expression signature of treatments or disease states. Microarrays provide limited quantitative data on gene expression, and on a single gene level they are limited in accuracy and reproducibility. Multigene transcriptional profiling, usually by real-time polymerase chain reaction (RT-PCR), lacks the breadth of microarrays but allows for the quantitative measurement of expression activity in a limited set of a priori identified genes. Next-generation sequencing technologies, such as RNA-Seq, provide analysis of the complete transcriptome, including noncoding microRNAs that may regulate expression. Unlike microarray-based technology, RNA-Seq is not limited to detecting transcripts corresponding to existing known genomic sequences, making RNA-Seq particularly adapted to the study of complex transcriptomics. However, because of the challenging and highly computationally intensive data analysis associated with RNA-Seq, microarrays remain the analysis method of choice in transcriptomics.[32]

Transcriptional profiling measures snapshots of gene activity at a particular point in time and therefore has the potential to identify rapid gene expression changes resulting from mind-body practices. However, this technique often lacks consistent findings, in part because of the diversity of expression across tissue types and the technical noise of the assay. For peripheral-blood studies, in which changes in the differential composition of leukocyte types may drive gene expression changes more than gene regulation within specific cell types, there is often insufficient discussion of the cell types involved in transcriptomic changes.[33] To handle these biases, some researchers recommend analysis on the level of gene ontology characteristics, as general trends in clusters of genes should provide a stable marker of expression that is robust to variation between trials.[33]

Proteomics is the quantification of protein in a given tissue sample, combining a technique to separate proteins (eg, 2-dimensional gel electrophoresis) with techniques to identify and quantify those proteins (eg, matrix-assisted laser desorption/ionization mass spectrometry). Proteomics is the next step in the study of biological systems after epigenomics and transcriptomics. As in transcriptomics, the proteome varies from cell to cell and from time to time because distinct genes are expressed in distinct cell types. Functional proteomics is a subfield that seeks to elucidate the specific cellular function of unidentified or novel proteins. Current proteomic methods are limited in scope; by some estimates, current methods are able to sample only a small portion of the available core proteome, consisting only of the most highly expressed proteins.[32]

Metabolomics is the study of the terminal downstream products of the genome, mainly small molecules and other low-molecular-weight end products of cellular processes. While the scope of metabolomic methods such as mass spectrometry and high-resolution nuclear magnetic resonance spectrometry is limited to a subset of the available small molecules in the cellular milieu, these analyses can be paired with quantitative measurements of small-molecule metabolites to provide a fuller picture of cellular activity.

In general, functional genomics approaches can be used in 2 ways. *Omics* approaches, because of their ability to look at the entire epigenome, transcriptome, proteome, or metabolome, are well suited for asking questions that are hypothesis generating, making them useful for exploring new areas. To make sense of the large quantities of data generated by omics methods, bioinformatics and systems biology approaches are frequently used to identify genes clustered into specific pathways, often centered around focus genes or hubs.[34] Using gene ontology groupings, it is also possible to ask targeted questions with omics data by analyzing data based on clusters of functionally related genes. Targeted studies, on the other hand, focus on the expression of specific genes or downstream markers. Because of the relative statistical power granted by the limited number of target genes or biomarkers, these targeted methods can provide more reliable quantitative data on specific differential expression than omics approaches. Targeted methods have a limited ability to detect patterns not contained within a priori hypotheses and can risk confirming false hypotheses by missing unmeasured mediators of effects.

MBT STUDIES IN THE LITERATURE

To assess the research applying functional genomics to MBTs, we searched MEDLINE for terms describing MBT (yoga, meditation, tai chi, stress management, stress reduction, and mind-body) and functional genomics (epigenetic, transcriptomic, gene expression, proteomic, and metabolomic). This search yielded 105 papers published between April

1999 and May 2014. Of these, 14 studies reported results of functional genomics analysis of humans practicing MBTs. One trial[35] did not appear in the initial search results but was included because of repeated mention in multiple reviews appearing in the results, bringing the total number of studies to 15. The studies included for analysis are described in the Table.

Study Designs

In the application of functional genomics to MBTs, the studies employed diverse designs and methods. Of the 15 trials, 4 were exclusively cross-sectional, comparing practitioners experienced in a given MBT to matched control subjects. Five trials used standard interventional designs with individuals or groups, assessing genomics outcomes before and after training in a specific MBT. Three studies solely employed a rapid-response method to assess changes in response to MBT by comparing samples from experienced practitioners before and after practice. Last, 3 studies used multiple methods: 1 was cross-sectional and interventional, 1 was cross-sectional and rapid-response, and 1 used all 3 methods. In the 2 studies using both cross-sectional and interventional methods, researchers compared naive practitioners to themselves and experienced practitioners. Of the 5 interventional-only studies, 3 used active control interventions, 1 used a waitlist control, and 1 had no controls, employing a quasiexperimental pre/post design. Figure 2 shows a detailed flow of study design types.

Study Populations

Study populations consisted primarily of healthy subjects. In 3 separate studies, however, populations were leukemia patients, women with current breast cancer diagnoses, and women with breast cancer histories reporting fatigue. In trials comparing experienced mind-body practitioners to controls, reported experience levels varied from 1-25 years. Many trials did not report the higher end of experience levels; 1 study reported estimated total lifetime practice hours as a measure of practitioner experience.

Intervention Lengths

Among interventional studies (alone and combined), 4 delivered 8-week interventions, 1 delivered a 10-week intervention, and 2 delivered 12-week interventions. Frequency and duration of practice for the interventions varied as well. Required at-home practice varied from 12-30 minutes daily; programs involving tai chi or yoga postures reported longer practice sessions, such as 90-minute sessions twice weekly. Experienced practitioners in cross-sectional studies reported higher levels of practice, with some studies reporting 60 minutes or more of average daily practice.

MBTs Used

The studies applied functional genomics to commonly practiced MBTs. The most popular modality was meditation, the sole component in 3 trials. Four trials reported interventions of mixed techniques, including meditation as the predominant component. Two studies reported breath regulation techniques, 1 study reported breath regulation and yogic postures, and another study reported yogic postures alone. Two studies featured tai chi practitioners, and 1 study investigated qigong. A small pilot trial reported the rapid response of 3 subjects to a session of clinical hypnosis. When categorizing these interventions between stationary/seated (eg, meditation and breath regulation) and dynamic practices (eg, yoga, tai chi, and walking meditation), 9 trials utilized exclusively stationary MBTs, while 6 utilized dynamic components. Regarding the origin of the traditions studied, 5 studies reported on yoga-derived interventions, 3 utilized Buddhist-derived practices, 3 utilized practices from traditional Chinese medicine, 1 reported on a European-derived technique (hypnosis), and 3 involved mixed techniques or practices of unknown origin.

Functional Genomics Methods

The majority of the studies used methods in transcriptomics. Thirteen studies assessed gene expression by partially or fully sequencing the transcriptome of peripheral white blood cells. Eight studies used microarray technologies alone, 2 studies used polymerase chain reaction (PCR) methods alone, 2 studies used microarrays in concordance with PCR methods, and 1 trial used microarray and ribonuclease protection assay. With regard to tissues sampled, 9 trials reported analyzing peripheral blood mononuclear cells (PBMCs) including lymphocytes and monocytes; 2 trials reported analyzing lymphocytes alone; 1 study reported analyzing neutrophils; and 1 study did not specify the type of leukocyte analyzed.

Of the remaining 2 studies, the first analyzed proteomic changes in peripheral blood with difference gel electrophoresis and Western blot techniques, and the second reported assessing DNA methylation in saliva samples.

COMMON FINDINGS

The majority of published research applying functional genomics methods to MBT is in the field of transcriptomics, supporting the correlation of transcriptomic, and thus epigenetic, changes with MBT. Despite the heterogeneity of the reviewed studies, the study results link MBTs to changes in cellular processes of

Table. Summary Characteristics of Mind-Body Therapy Trials Reviewed

Author, Year	Mind-body Modality	Total Subjects	Type of Subjects	Study Type	Training Time	Practice Frequency	Follow-up	Control Method	Cell Tissue Type	Assay Method
Li et al, 2005[36]	Qigong	12	Falun Gong practitioners with 1-5 years experience, matched naive controls	Cross-sectional	1-5 years	60-120 minutes/day	None	6 experienced practitioners, 6 matched naive controls	Peripheral blood neutrophils	Microarray, replication protein A
Sharma et al, 2008[37]	Sudarshan Kriya breath regulation	84	Sudarshan Kriya practitioners, naive controls	Cross-sectional	>1 years	>60 minutes/day	None	42 experienced practitioners, 42 naive controls	Peripheral blood lymphocytes	RT-PCR
Kumar and Balkrishna, 2009[38]	Pranayama breath regulation	8	Chronic lymphocytic leukemia patients	Cross-sectional	Not reported	Not reported	None	Experienced practitioners and nonpractitioner controls (number of each not reported)	PBMCs	Microarray
Ren et al, 2012[39]	Tai chi chuan	500	Women ages ≥45	Cross-sectional	>3 years	60 minutes/week	None	237 experienced practitioners, 263 matched controls	Saliva	MALDI-TOF MS methylation analysis
Antoni et al, 2012[40]	Cognitive-behavioral stress management (CBSM)	79	Women with stage 0-III breast cancer	Interventional (group)	10 weeks	Not reported	6 and 12 months postintervention	45 CBSM, 34 active control receiving 1-day intervention	PBMCs	Microarray, qRT-PCR
Bower et al, 2014[41]	Iyengar yoga	31	Stage 0-II breast cancer survivors with fatigue	Interventional (group)	12 weeks	90 minutes/twice weekly	3 months postintervention	16 intervention group, 15 active health education controls	PBMCs	Microarray
Creswell et al, 2012[42]	Mindfulness-based stress reduction	40	Healthy normal older adults (55-85 years)	Interventional (group)	8 weeks	30 minutes/day	None	20 intervention group, 20 waitlist controls	PBMCs	Microarray
Yang et al, 2010[43]	Tai chi chuan	3	Healthy adults without prior experience	Interventional (group)	12 weeks	60 minutes/thrice weekly	None	None	Peripheral blood	DIGE, Western blot for validation
Black et al, 2013[44]	Kirtan Kriya meditation	39	Caregivers of family with dementia	Interventional (individual)	8 weeks	12 minutes/day	None	23 intervention group, 16 active controls receiving relaxing music intervention	PBMCs	Microarray
Rossi et al, 2008[35]	Clinical hypnosis	3	Highly susceptible hypnotic subjects	Rapid response	Not reported	Not reported	None	None	Peripheral blood leukocytes	Microarray
Qu et al, 2013[45]	Sudarshan Kriya and yoga postures	10	Male Sudarshan Kriya practitioners	Rapid response	1.5 months to 5 years	Not reported	None	Within-subject; compared alternate days of yoga practice and simply relaxing	Peripheral blood lymphocytes	Microarray, qPCR
Ravnik-Glavač et al, 2012[46]	Meditation	2	Male meditators	Rapid response	>23 years	Not reported	None	None	PBMCs	Microarray
Dusek et al, 2008[14]	Relaxation response elicitation training	39	Long-term practitioners, matched naive controls	Cross-sectional, interventional (individual)	8 weeks	20 minutes/day	None	19 experienced practitioners and 20 matched naive controls; pre/post 8 weeks of training for naive group	PBMCs	Microarray

Table. Continued.

Author, Year	Mind-body Modality	Total Subjects	Type of Subjects	Study Type	Training Time	Practice Frequency	Follow-up	Control Method	Cell Tissue Type	Assay Method
Kaliman et al, 2013[47]	Mindfulness meditation	40	Experienced meditators, matched naive controls	Cross-sectional, rapid response	>3 years	>30 minutes/day	None	19 experienced practitioners, 21 matched naive controls	PBMCs	qPCR
Bhasin et al, 2013[48]	Relaxation response elicitation training	52	Long-term practitioners, matched naive controls	Cross-sectional, interventional (individual), rapid response	8 weeks	20 minutes/day	None	26 experienced practitioners and 26 matched naive controls; pre/post 8 weeks of training for naive group	PBMCs	Microarray

DIGE, difference gel electrophoresis; MALDI-TOF MS, matrix-assisted laser desorption-ionization time-of-flight mass spectrometry; PBMCs, peripheral blood mononuclear cells; qPCR, quantitative polymerase chain reaction; qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction.

inflammation, apoptosis, ubiquitin-dependent protein catabolism, and telomere regulation.

Regulation of Inflammatory Signaling in the Nuclear Factor-Kappa B Pathway

Among the common patterns of gene expression, the most consistent is the downregulation of inflammatory signaling in the nuclear factor-kappa B (NF-κB) pathway. NF-κB, a gene transcription factor involved in modulating immune function and inflammatory response, consists of a group of similar proteins (NF-κB 1, NF-κB 2, RelA, RelB, c-Rel) related by means of a DNA-binding/dimerization domain. Dysregulation of the NF-κB pathway has been linked to inflammation, chronic diseases, and the development and proliferation of cancers.[49-51] Miller et al described increased NF-κB signaling and a blunted glucocorticoid response as the hallmark biological sign of chronic stress in humans.[52] NF-κB is critically implicated in a proinflammatory signaling pathway that includes upstream regulators (such as tumor necrosis factor alpha [TNFα], receptor-interacting serine/threonine-protein kinase 1 [RIPK1], mitogen-activated protein kinase kinase kinase [MAP3K], and IkappaB kinase [IKK]),[53] downstream targets (such as cytokines like interleukin-1beta [IL-1β] and TNFα), and inflammatory enzymes (such as cyclooxygenase-2 [COX2]).[51]

Interference at many of these upstream levels can influence later gene expression and inflammatory response.[51]

Downregulation of the NF-κB pathway as a result of MBT has been documented in multiweek interventions[40,41,44,48,54] and in rapid-response gene transcription following MBT.[47,48] In our group's initial study in this area, gene ontology analysis revealed an overrepresentation of genes related to the NF-κB cascade in genes downregulated in healthy adults following 8 weeks of meditation training.[54] Antoni et al reported that specific NF-κB target cytokines were differentially downregulated following a 10-week comprehensive stress management program compared to controls.[40] At 6- and 12-month follow-up, bioinformatics analysis revealed reduced activity of NF-κB, indicated by significant underrepresentation of genes targeted by NF-κB transcription factors in genes differentially upregulated in the intervention group. Black et al found similar reduced expression in genes with NF-κB response elements following an 8-week training in Kirtan Kriya meditation.[44] In a 2012 study of a mindfulness-based stress reduction (MBSR) intervention for older adults, Creswell et al found increased expression of NF-κB–related genes at baseline in the individuals who reported greater loneliness.[42] The MBSR intervention reversed this trend, effecting reduced activity in the NF-κB pathway

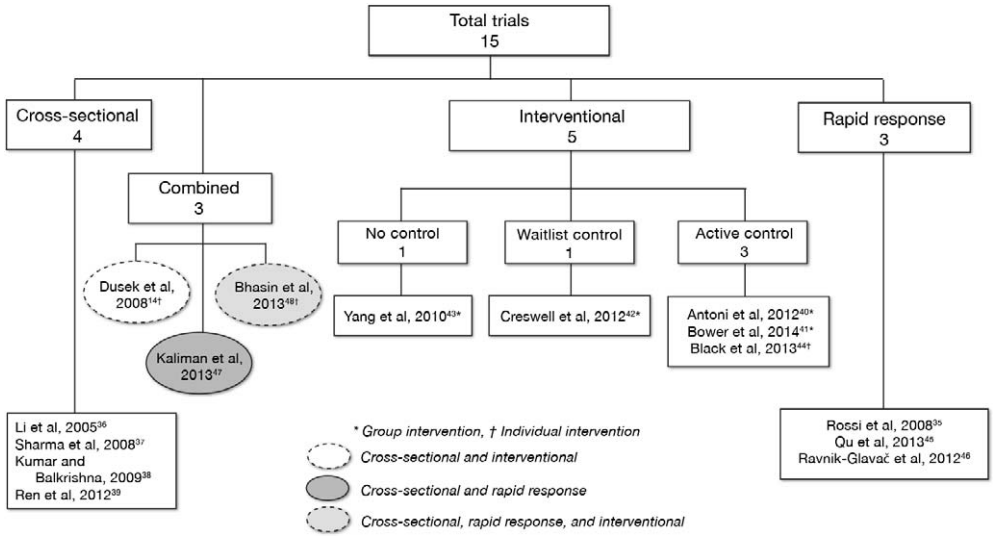

Figure 2. Trials included in this review by study design type, use of controls, and time course of effects measured.

in the MBSR group relative to waitlist controls. In a 12-week randomized controlled trial of an Iyengar yoga intervention for stage 0-II breast cancer survivors with treatment- or disease-related fatigue, Bower et al found reduced activity of NF-κB, along with increased activity of the antiinflammatory glucocorticoid receptor at both endpoint and 3-month follow-up.[41] NF-κB–related expression changes have been implicated even in rapid responses to MBT. Our group identified downregulation of NF-κB and its interactants in both experienced MBT practitioners and newly trained individuals relative to baseline.[48] In a targeted analysis of inflammatory, circadian, and histone modification mechanism gene expression, Kaliman et al found that meditators had significantly downregulated expression of receptor-interacting serine/threonine-protein kinase 2 (RIPK2) and COX2 during the course of an 8-hour meditation retreat.[47] RIPK2 and COX2 are component genes in NF-κB–dependent pathways.

Regulation of Proinflammatory Cytokine Production

MBTs may target a specific domain of NF-κB–related activity: the regulation of certain proinflammatory cytokines that regulate inflammatory processes. Interleukins, interleukin-6 (IL-6) in particular, are markers of inflammation linked to chronic stress;[55] thus, it makes conceptual sense that MBTs target them. Previous trials have found a reduction in circulating levels of IL-6 in plasma following a 12-week walking meditation intervention[56] and short-term yoga interventions,[57-60] as well as reduced IL-6 levels in expert yoga practitioners compared to naive controls.[61] One trial of a 10-week multimodal mind-body program identified downregulation of the genes coding IL-1β and IL-6.[40]

However, the link between MBTs and cytokine production is not yet clear. While studies in the mind-body literature report downregulation of the NF-κB pathway as the dominant genomic trend, this effect is not seen across all studies. Kaliman et al[47] did not see differences in inflammatory gene expression between long-term meditators and controls at baseline. Early cross-sectional studies did not identify a change in NF-κB regulation,[36,37] and NF-κB was not implicated in a 2013 trial of rapid-response gene expression following a 2-hour Sudarshan Kriya yoga routine.[45] Other researchers found no alteration in production of IL-6 or the related IL-8 following MBSR training[42,62-64] or training in compassion meditation.[65] A 2011 trial failed to find differences in interleukin expression following tai chi exercise,[66] while another indicated a possible link between qigong and production of IL-6–secreting cells.[67] Bower et al, whose genomics analysis revealed overall NF-κB downregulation, found no change in IL-6.[41] One trial of MBSR revealed increased production of the proinflammatory interferon gamma immediately after the intervention, with significant declines relative to baseline at 6- and 12-month follow-up.[68] Another group reported no change in IL-6 serum concentration following a day of intensive mindfulness meditation in experienced meditators.[47]

Regulation of Apoptotic Processes

Apoptosis is the process of programmed cell death seen in all multicellular organisms. Regulation of these apoptotic processes through regulators such as B-cell lymphoma 2 (Bcl-2) and heat-shock protein

groups such as 70 kilodalton heat-shock proteins (Hsp70) may be another common target of MBT. This relationship appears complicated: upregulation of antiapoptotic genes in PBMCs has been demonstrated in trials of yogic breath regulation;[37,38] an early trial of gene expression in neutrophils of qigong practitioners found significant downregulation of antiapoptotic Bcl-2 and B-cell lymphoma-extra large (Bcl-xL) but upregulation of antiapoptotic Hsp70.[36] A pilot study of gene expression changes in advanced meditation practitioners during practice demonstrated rapid downregulation of antiapoptotic genes,[46] although the small sample size (n=2) limits interpretation of these data. In our group's studies,[48,54] we observed significant changes in expression of genes related to apoptotic regulation in cross-sectional analysis between experienced MBT practitioners and naive controls and prospectively during an 8-week intervention.

Regulation of the Ubiquitin-Dependent Protein Catabolic Pathway

Another potential genomic target of mind-body interventions is the ubiquitin-dependent protein catabolic pathway that regulates the breakdown of proteins by the proteasome responsible for ridding cells of unneeded or damaged proteins. Through gene ontology analysis, our group found this pathway to be downregulated in cross-sectional and prospective analyses.[54] Downregulation of ubiquitin-dependent protein catabolism has also been reported in qigong practitioners and experienced meditators.[36,46]

PARALLELS AND POTENTIAL TARGETS

To better understand genomic commonalities among these diverse practices, it is important to draw comparisons of functional genomics changes brought about by MBTs to other practices—for example, exercise and massage—that share some characteristics and produce similar changes in gene expression. On the transcriptome level, exercise interventions show some similarities with MBTs. A 2011 trial of resistance exercise indicated differential expression of the NF-κB signaling pathway,[69] and a 2-month aerobic exercise intervention reduced age-related upregulation of NF-κB and the related cytokine TNFα.[70] However, rapid-response studies of aerobic and anaerobic exercise reveal upregulation of inflammation-related gene pathways,[71-75] indicating a paradoxical response with short-term upregulation of inflammatory pathways leading to long-term downregulation. This response stands in contrast to the progressive pattern of rapid-response signaling seen in MBTs[48] that correlates positively to long-term effects. Denham et al found that multimonth exercise interventions significantly altered epigenetic mechanisms in peripheral blood leukocytes,[76] although whether parallel changes occur with MBTs remains to be determined, as the only MBT study to analyze methylation featured tai chi, a form of light exercise.[39]

Massage therapy has been demonstrated to reduce psychological stress[77] and to influence immune function[78,79]—both effects commonly seen with MBTs—and may bear genomic similarities to MBTs. A pilot trial found that a 40-minute session of traditional Japanese massage therapy effected PBMC upregulation of genes related to immune response compared to a 40-minute resting control period in 2 healthy women.[80] Bittman et al found that participation in a creative music-making group significantly reversed proinflammatory cytokine expression activity.[81] These studies suggest shared gene expression changes that may be related to a common signature around perceived stress reduction.

Finally, there is a growing body of research regarding gene expression and other functional genomics technologies in acupuncture. While commonly cited findings overlap with some potential targets of MBT, such as regulation of apoptosis, direct comparisons are difficult, as acupuncture trials often target pain-related genes because of their clinical relevance for acupuncture.[82]

DISEASE AND TREATMENT CONDITIONS

MBTs have been used to treat a wide variety of diseases, among them irritable bowel syndrome,[15] hypertension,[6,14] insomnia,[6,16] and depression.[19,20,21] By comparing the gene expression changes associated with disease states to the gene expression changes elicited by MBTs, it is possible to identify diseases in which MBTs counterregulate gene pathways central to the illness (ie, downregulate or upregulate genes typically upregulated or downregulated, respectively, by the disease process). Such elucidation of counterregulated gene expression signatures may improve knowledge of the mechanisms of MBTs and suggest the use of MBTs in novel treatment contexts. Two studies suggest that the genomic signature of chronic stress in humans is driven primarily by upregulation of the NF-κB pathway, coupled with blunted glucocorticoid signaling.[52,83] This stress signature shares common domains with, but runs opposite to, the signature of MBT. This stress signature shows strong overlap with the gene expression profile of traumatic stress seen in PTSD that involves significant upregulation of the NF-κB pathway[84] and apoptotic modulation,[85] thus overlapping significantly with domains of the MBT signature.

Given the link between MBT, inflammation, and immune response, cancer is another potential epigenetic target of MBT. Our group is investigating

hypotheses stating that the MBT-associated gene signature runs counter to gene expression changes seen in the progression of hematological cancers, such as multiple myeloma.[86] Studies have demonstrated MBT-related gene expression changes in patients with breast cancer[40] and leukemia,[38] although no connection to disease status or progression has been observed. While the impact of MBT on disease progression is modest in aggregate, functional genomics is useful in identifying individual propensities to improve through treatment, as well as propensities to develop diseases, and may prove beneficial in understanding treatment response to MBT.

BEYOND THE TRANSCRIPTOME

Epigenomics

Few studies look directly at changes to the epigenome through MBTs. Histone protein modifications in brain tissue in response to stress take effect rapidly,[87] so changes related to stress-reducing practices like MBT may be similarly fast acting. Selective downregulation of histone deacetylase genes HDAC2, HDAC3, and HDAC9 has been observed following a single 8-hour meditation retreat, indicating the likelihood of rapid epigenetic activity following MBT.[47] In a cross-sectional study of female tai chi practitioners, Ren et al found evidence of long-term epigenetic changes resulting from practice.[39] Of 60 a priori targeted age-related CpG sites, 6 sites (17p 7, Xp13 1, Rad50 2, Rad50 10, G6PD 6, and G6PD 7) showed significant differentiation in methylation between practitioners and controls, with some changes showing age-related variation. While the clinical impact of these changes is unknown, methylation analyses in MBT interventions can provide insight into whether methylation changes are observable during short-term therapeutic intervals or only after years of practice. Ultimately, correlation of DNA methylation changes with simultaneous gene expression profile analyses could identify causal relationships between DNA methylation and gene expression changes following MBT.

Metabolomics and Proteomics

To our knowledge, no published work exists regarding the metabolome in MBTs. The field of MBT and proteomics is also underdeveloped, with only 1 paper published to date. In a trial of 12 weeks of tai chi training, healthy normal practitioners increased expression of complement factor H while decreasing levels of complement factor B, indicating possible delays in age-related physical decline.[43]

Telomere Maintenance

Using targeted proteomics methods, MBTs have been linked to changes in telomere maintenance through regulation of the protein telomerase. Telomeres that cap the ends of DNA strands and prevent their deterioration gradually shorten with age[88] and are subject to further damage because of stress.[89] Although telomere maintenance is not strictly included in functional genomics per se, it bears discussion here because telomere maintenance is an active target of investigation among MBT researchers. Truncated telomeres in PBMCs have been linked to lowered telomerase activity, indicate higher mortality,[88] and may serve as a risk factor for chronic disease.[88,90] Ornish et al[91] offered the first indication that lifestyle was linked to telomerase in their study of a comprehensive diet, relaxation, and exercise program for low-risk prostate cancer patients. In a 5-year follow-up of the original cohort, Ornish et al found that while telomeres of controls had decreased in length, telomeres of those in the lifestyle modification group had grown longer, with greater lifestyle change linked to greater increases in length.[92]

Similar positive changes in telomere maintenance have been seen in studies of MBT. In their study of Kirtan Kriya meditation in dementia caregivers, Black et al[44] found that meditators had significant increases in telomerase activity during the 8-week study period compared to controls (43% vs 4%). Increases in telomerase activity have also been observed following a 4-month qigong intervention[93] and for experienced meditators following an intensive 3-month meditation retreat.[94] A further group reported increased telomerase activity for both an MBSR group and waitlist controls.[95] In addition, our group found that long-term practice of MBT was linked to upregulation of genes related to telomere maintenance.[48] Effects of MBT on telomeres may be subject to specific characteristics of both the practice and the practitioner. Hoge et al[96] have reported longer telomere length in female long-term practitioners of loving-kindness meditation compared to matched, meditation-naive controls, with no significant effect in male practitioners.

LIMITATIONS, CHALLENGES, AND MOVING FORWARD

While several general trends can be deduced from functional genomics outcomes with MBT, the results reported thus far must be interpreted with caution. For example, while downregulation of the NF-κB pathway is the dominant genomics trend in the mind-body literature, several studies did not report NF-κB–related downregulation.[36,37,45] Such variation in results may be because of the diversity of study designs and research questions chosen by individual research groups in this developing field. In a 2013 case, Qu et al used a within-subjects model examining experienced yoga practitioners on a retreat.[45] While this design reduced potential confounds, the short time

window did not allow for analysis of long-term gene expression changes that may have resulted from subjects' years of practice, highlighting the difficulty in comparing these results with other trials.

In general, greater coordination between groups in assembling similar study designs and questions will be critical for moving the field of mind-body genomics forward. Uniformity in sample populations, intervention lengths, and genomics methodologies will ensure easier comparisons among trials. Another key development that we recommend is supplementation of microarrays with more advanced techniques. As mentioned above, several genomics techniques, such as RNA-Seq and DNA methylation sequencing, have yet to be fully utilized in mind-body research. Greater use of these techniques will accelerate our understanding of MBT-related genomics changes.

In addition to these broad recommendations, the field may now be developed enough to address several significant limitations in the current body of literature. For example, most of the studies included in this review are small trials, limiting the strength of conclusions. Our review of the literature found only 1 study with more than 100 subjects, the findings of which are difficult to compare to those of other studies as it is the only trial to target DNA methylation.

Several of the studies covered in this review used active control conditions[40,41,44] but not all adequately controlled for attention. In 1 study, the active group met weekly for 10 weeks; the control group received a 1-day intensive intervention.[40] Within the field of mind-body medicine, there have been recent advancements in using health or stress education programs as active controls for meditation interventions.[96] Providing controls with a daily activity, such as reading or listening to music or audiobooks, can control for MBT practice time and the presence of a daily routine. With the exception of a 2014 trial,[41] none of the studies included in this review used this design for mind-body genomics analyses. We recommend that, when applicable, future studies feature adequately sized control groups receiving non-MBT interventions, such as stress education.

As mentioned earlier, the diversity in specific cell types used in transcriptomic analysis deserves careful consideration. Individual white blood cell types vary in their expression of mRNA, and thus observed changes in expression for whole blood may be driven in part by alterations in leukocyte composition or by 1 cell population rather than indicating changes in expression across multiple cell types.[33] We recommend that future trials include an examination of specific peripheral blood cell lines; in addition, the investigation of non-PBMC tissues (such as the prostate biopsies in men with prostate cancer noted above[91,92]) would greatly expand our understanding of the effect of MBT on different tissue types and in different disease states.

Heterogeneity of subject populations creates further contextual challenges. Some trials reviewed above investigated patients with specific disease states, while others opted for healthy subjects. Comparisons between these populations should be made cautiously, as certain diseases may predispose subjects to show blunted or heightened genomic responses to MBTs, or disease-related genomic shifts may hide subtle MBT-related responses. While we advocate for trials researching the effect of these practices in clinical populations, well-powered studies with healthy subjects are also needed to determine a baseline genomic signature with greater confidence.

Additional research to discern the length of practice that is needed for long-term benefit from MBT would be helpful. Cross-sectional studies of long-term practitioners have clear methodological limitations, and long-term longitudinal studies are practically difficult and often prohibitively expensive. A research design that has been suggested to address these issues is temporary practice pause then resumption (TPPR).[97] TPPR pairs experienced practitioners against each other as controls, as some practitioners are randomized to halt their mind-body practice for an observed interval. The TPPR model might be a novel and cost-effective method to address several issues at once: the possibility of a confounding characteristic (personality or otherwise) that influences someone to choose to practice a mind-body technique long term, the potential for enduring epigenetic changes to be overlooked in rapid-response study designs, and the difficulty in determining how much practice experience is necessary to evoke the long-term response signature.

Another point of consideration is the heterogeneity of MBT practices themselves. The literature contains trials of both sedentary practices (such as meditation or yogic breath regulation) and dynamic practices (such as yoga asanas and qigong) that may differ in objects of mental attention, levels of physiological arousal, and general goals of practice. Even within meditation, the most common practice encountered in this review, there may be considerable variation. A 2013 trial of MBSR that included training in multiple mind-body techniques, such as yoga, body scans, and sitting mediation, found differential levels of circulating inflammatory markers depending upon which technique was used most frequently for daily practice.[62] Furthermore, while we have used the broad term MBT to describe these practices, many studies did not involve a specific therapeutic context. Further studies may need to account for social effects

within these various techniques and determine specific benefits that may be associated with individual or group mind-body practice. In addition, comparisons between mind-body group interventions and group-based active controls may be able to differentiate effects that are specific to MBTs from those common to group therapies.

The frequency and time of MBT practice in research interventions also deserve consideration. Current protocols for short-term interventions favor 8- to 12-week models with weekly visits and daily practice; these have demonstrated clear clinical benefits. However, the specific daily prescription varied among studies covered here; for example, 1 study involved practice only twice weekly.[41] For the trials included in this review, practice fidelity of study participants was either not recorded or not reported, so conclusions cannot be drawn about the importance of intervention adherence for genomic determinants. MBT-related gene expression may be a biomarker suited to a dose-response relationship, and future studies should strive to accurately record subjects' frequency and duration of practice.

Despite the heterogeneity of mind-body practices in the studies reviewed, we hypothesize that these practices share a common underlying physiology—just as different forms of exercise share a common set of underlying physiological changes whether one is running, kayaking, biking, or swimming. Needless to say, different MBTs cannot be identical in their effects on the brain and the body. Still, the ability of varied mind-body practices to demonstrate similar physiological effects[98] and to effect correspondingly similar clinical changes[6,22,99] is evidence that diverse MBTs may take effect through shared molecular mechanisms and may share a core genomic signature with reliably regulated components.

CONCLUSION

The field of mind-body genomics is still in its infancy, but the body of literature is growing, particularly in transcriptomics. The heterogeneity of practices and research methodologies speaks to the general diversity within mind-body medicine, and many trials are limited by methodological challenges. Preliminary results indicate the presence of an underlying common genomic signature shared across diverse practices, focused on key hubs of expression such as downregulation of the NF-κB proinflammatory pathway. Large-scale randomized controlled trials will confirm or reject the other signature hubs outlined above, including regulation of apoptosis, telomerase function, and ubiquitin-dependent catabolism. In addition, the exciting expansion of MBT studies into epigenomics, using histone modification analysis and DNA methylation sequencing technologies, will greatly increase our understanding of epigenetic changes following MBT and allow a fuller picture of how MBT may have lasting effects.

As increased resources are invested in functional genomics studies in diseases known or hypothesized to be stress-related and thus amenable to mind-body approaches, studies will be able to determine specific pathways of action and identify new potential targets for these interventions. This body of work will provide greater understanding of the profound links between the mind, the body, and the environment and inform clinical practice by providing doctors and their patients with an understanding of how to prevent disease, treat disease, and promote health using MBTs.

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