ORIGINAL ARTICLE

A Pilot Study of Positive Expectations and Focused Attention via a New Protocol for Optimizing Therapeutic Hypnosis and Psychotherapy Assessed with DNA Microarrays: The Creative Psychosocial Genomic Healing Experience

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We extend the use of DNA microarrays to explore a new psychotherapeutic therapeutic protocol, The Creative Psychosocial Genomic Healing Experience, an easy-to-learn approach to facilitating therapeutic hypnosis, psychotherapy, rehabilitation, meditation, and pastoral counseling. This pilot study assessed the hypothesis that a top-down creatively oriented positive human experience can modulate gene expression on the molecular level. A DNA microarray data analysis of the white blood cells of three human subjects was performed immediately before, one hour after, and 24 hours after The Creative Psychosocial Genomic Healing Experience. We documented changes in the expression of 15 early response genes within one hour that apparently initiated a further cascade of 77 genes 24 hours later. This could provide the mind/molecular genomic foundation of new therapeutic models for optimizing human consciousness, health, and well being via therapeutic hypnosis, psychotherapy, pastoral counseling, and psychiatry. This proof-of-principle pilot study now requires cross validation with more subjects with a variety of diagnostic classifications to document the validity and reliability of using DNA microarrays to assess our new creative psychosocial genomic therapeutic protocol in a variety of cultures. **(Sleep and Hypnosis 2008;10(2):39-44)**

Key words: Creative Experience, DNA microarray, gene expression, therapeutic hypnosis.

INTRODUCTION

In the past decade DNA microarray technology has made it possible to measure the expression levels of many thousands of genes simultaneously. This novel experimental approach has revolutionized research in molecular biology and become a new standard in personalized medicine (Eisen et al. 1998). Recent research has documented the use of DNA microarrays for assessing therapeutic responses to psychological relaxation and meditative practices on the molecular-genomic level (Dusek et al., 2008; Rossi, 2002, 2004, 2007; Rossi, 2005/2006). This has lead to calls for further research on the pathways of psychotherapeutic

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processes on all levels from mind to gene (Abbott, 2008; Nestler, 2008; Rossi et al., 2006). In this pilot study we use DNA microarrays to assess a new therapeutic protocol, The Creative Psychosocial Genomic Healing Experience, is a relatively brief, easy-to-learn process for facilitating a wide range of therapeutic approaches such as therapeutic hypnosis, psychotherapy, rehabilitation, meditation, and pastoral counseling (Rossi, 2005/2006; Rossi and Rossi, 2008a and 2008b).

MATERIAL and METHODS

Three highly susceptible hypnotic subjects, one male and two females, experienced therapeutic hypnosis following the new protocol called, "The Creative Psychosocial Genomic Healing Experience" established by Rossi (2004).

Peripheral blood, about 10 ml, was collected immediately before, within one hour of therapeutic treatment (the length of each treatment depended by the subject, but never went more than one hour). A total of 6 blood samples were employed to purify total RNA from leukocytes (the nucleated part of peripheral white blood cells) using the kit LeukoLOCK(TM) Total RNA system following the instruction supplied by the manufacture (Ambion, USA).

The amount of total RNA extracted from each blood sample was quantified using the NanoDrop ND-1000 photometer (Wilmington, DE). The purity of RNA samples was determined based on the ratio of spectrophotometric absorbance of the sample at 260 nm to that of 280 nm (A260/A280). However not all the RNA samples resulted pure enough (A260/A280 ratio of 1.8) to go through the microarray procedure, in fact protein contamination was still present in some of the samples. A further purification step by means of one phenol chloroform and one chloroform treatment was necessary to eliminate the contaminants.

Total RNA of each purified sample was further quantify using the NanoDrop apparatus and its integrity was ascertained by means of electrophoresis in agarose gels followed by ethidium bromide staining on the ribonucleic acid. Around 2.5 μg of total RNA was delivered to the MicroCRIBI Service (University of Padova, Italy) for microarray analysis. MicroCRIBI Service performed the microarray analysis on 21,329 - 70mer oligonucleotides (Operon version 2.0) designed on Human Unigene clusters.

For each sample, 1.0 μg of total RNA was reverse transcribed and labelled using Amino Allyl cDNA Labeling Kit (Ambion, USA) following the manufacture instruction. Cy3/Cy5 was from Amersham Biosciences (Amersham, United Kingdom). Cy3/Cy5 dye incorporation into aRNA yielded incorporation rates of 30 to 60 dye molecules per 1000 nucleotides by spectrophotometric analysis, as requested by the manufacturer.

The microarrays were scanned with a two-channel confocal microarray scanner (ScanArray# Lite, Perkin Elmer,USA) using its dedicated software (ScanArray Express 3.0.0.,Perkin Elmer). The laser power and the photomultiplier tube (PMT) were set between 70% and 80% of maximum. The excitation/ emission settings were 543/570 nm for Cy3 and 633/670 nm for Cy5. After laser focusing and balancing of the two channels, scans were conducted at a resolution of 5 μm. For any scan, two separate 16-bit TIFF images were produced. Data were normalized by ScanArray Express using the LOWESS (Locally Weighted Regression Scatter Plot Smoothing; Cleveland, 1979) algorithm

After normalization, data from each slide were split in two, by using Microsoft Excel, since each probe is spotted twice. Thereafter, each spot value was considered to be independent and subjected to SAM (Significance Analysis of Microarrays; Tusher et al., 2001) analyses.

Since each comparison (S1/S1-1h, S2/S2-1h, S3/S3-1h,) was repeated at least twice, there were at least four values for each gene to be used in the SAM analyses.

Lists of genes with significant changes in

expression among at least two experimental samples were identified at delta values that gave a false discovery rate (FDR) of 0%.

The possible role played by therapeutic hypnosis via "The Creative Psychosocial Genomic Healing Experience" on three subjects in up-regulating gene expression in leukocytes in the peripheral blood was investigated. To accomplish this, transcriptome changes were first monitored in untreated subject, just after the treatment. The transcriptome variations were analysed by means of the Operon Human Genome Oligo Set Version 2.0 platforms. The effect of positive and creatively oriented therapeutic hypnosis immediately after the session was investigated using a direct comparison experimental design, with four repetitions of which one was a dye swap. As each probe was spotted twice on human array, the following SAM (Significance Analysis of Microarrays, Tusher et al., 2001) analysis was performed on a dataset of eight values for each gene.

Experimental Design

The effect of positively oriented therapeutic hypnosis via the administration of our Creative Psychosocial Genomics Healing Experience on gene transcription was monitored and the subject's measurements before the treatment were the common references. Each comparison (S1/S1-1h, S2/S2-1h; S3/S3-1h) was repeated at least twice, so that at least four values for each gene were used in the subsequent SAM analyses. The dataset was tested with SAM using a 'one class' study design. This analysis was carried out on the following three subgroups of data: S1/S1-1h, S2/S2-1h; S3/S3-1h. Using a delta of 0.2 and a median false discovery rate (FDR) of 0.00%, the analysis yielded 3207 genes as differentially expressed, six groups of genes were selected (the up- and down-regulated for each of the three subgroups of data). By crossing these data, it was possible to show the different up- and down-regulating effects.

RESULTS

DNA microarray results on the three subjects in response to the therapeutic protocol within one hour after the treatment indicated that expression of 15 early response genes were up-regulated between 1.2 and 1.8 folds and no single gene was down-regulated. The list of the up-regulated genes is presented in Table 1.

Table 1. The Gene Bank Accession, Gene Symbol, Gene Description and results in fold changes in response to therapeutic hypnosis.

GB_accession	Gene_Symbol	Description	Fold changes
AK057104		Homo sapiens cDNA FLJ32542 fis, clone SMINT2000537	
		Sodium-coupled neutral amino acid transporter 2	1,777714817
NM_000329	RPE65	Retinal pigment epithelium-specific protein (65kD)	1,664647867
AK055997		Homo sapiens cDNA FLJ31435 fis, clone NT2NE2000612	
		Ring Finger protein 165	1,617968537
AK056729		Homo sapiens cDNA FLJ32167 fis, clone PLACE6000450	
		Serpin B Proteinase Inibitor	1,596523872
NM_001074	UGT2B7	UDP glycosyltransferase 2 family, polypeptide B7	1,578875081
BC018130	F2RL1	Coagulation factor II (thrombin) receptor-like 1	1,506199199
NM_030824	FLJ14356	Hypothetical protein FLJ14356 zinc finger protein 442	1,469687506
NM_021122	FACL2	Fatty-acid-Coenzyme A ligase, long-chain 2	1,380622376
NM_004126	GNG11	Guanine nucleotide binding protein 11	1,372082479
NM_020980	AQP9	Aquaporin 9	1,366899043
NM_001186	BACH1	BTB and CNC homology 1, basic leucine zipper transcription factor 1	1,330834867
NM_002921	RGR	Retinal G protein coupled receptor	1,312291611
NM_024911	FLJ23091	Hypothetical protein FLJ23091	
		G protein-coupled receptor 177 Isoform 1 and Isoform 2	1,274787709
NM_000860	HPGD	Hydroxyprostaglandin dehydrogenase 15-(NAD)	1,224585804
NM_002110	HCK	Hemopoietic cell kinase	1,190732546

DISCUSSION

The major limitation of this pilot study was the small number of subjects and the lack of appropriate controls. The lack of statistical power in this pilot study, due to the limited number of treated subjects, for example, does not enable us to assess the degree to which our results coincides with previous research supporting the hypothesis that therapeutic hypnosis could modulate gene expression (Lichtenberg et al., 2000, 2004). Well funded major research studies in psychiatric genetics utilizing DNA microarrays typically include as many as 2,000 to 20,000 subjects (Abbott, 2008). Our results, however, suggest that this pilot study provides documentation consistent with the hypothesis that our new therapeutic protocol, The Creative Psychosocial Genomic Healing Experience, may modulate gene expression in human white blood cells.

These preliminary findings suggest that positive expectation via The Creative Psychosocial Genomic Healing Experience, a new protocol for facilitating therapeutic hypnosis, generated the up-regulation of 15 early response genes within one hour.

The expression of these early response genes apparently initiated a larger cascade of gene expression 24 hours later. This unexpected finding may have important implications for the role of time and post-hypnotic suggestion in therapeutic hypnosis and many other psychological experiences. We are currently investigating the unexpected finding that a gene associated with bipolar disorder may be over expressed in response to our new protocol of therapeutic hypnosis.

We propose that the genes expressed in response to this new protocol may be related to a variety of functions associated with stress (Dusek et al. 2008), cognition and dreaming (Riberio et al., 2007), and psychiatric conditions (Tsankova et al., 2007). Suggestions for further research in this area have been recommended previously (Kustra et al., 2006; Nestler, 2008; Nuzzo, 2008).

We introduced a new therapeutic protocol, The Creative Psychosocial Genomic Healing Experience, for assessing the contribution of positive expectation, focused attention, therapeutic hypnosis, and psychotherapy to stress reduction and mind-body healing (Rossi, 1986/1993, 2002, 2004, 2007; Rossi and Rossi, 2008b). A salient feature of this new protocol is that it was found to be more acceptable, with high face validity for the subjects who experienced it and the psychiatrist who administered it, as a positive therapeutic process in contrast to the more research oriented classical scales of measuring hypnotic susceptibility, which have been questioned regarding their appropriateness for therapeutic applications (Fromm & Shor, 1972 Wester and Sugarman, 2007).

Our new protocol, The Creative Psychosocial Genomic Healing Experience, however, now requires standardization in relation to the classical assessments of therapeutic hypnosis such as the Stanford Hypnotic Susceptibility Scale (Hilgard, 1965) and the Harvard Group Scale of Hypnotic Susceptibility (Shor and Orne, 1978) as well as the more general evaluation of consciousness and focused attention with objective measures such as the Tellegen Absorption Scale (Tellegen, 1981, 1982, 1992; Tellegen & Atkinson, 1974).

The successful utilization of this new Creative Psychosocial Genomic Healing Experience protocol for assessing research on humans with DNA microarrays may foreshadow a new psychosocial genomic paradigm to facilitate a “top-down” therapeutic approaches from mind to gene (Rossi, 1986/1993, 2007; Rossi and Rossi, 2008a & b). This could provide the mind/molecular genomic foundation of new therapeutic models for optimizing human consciousness, health, and well being via therapeutic hypnosis, psychotherapy, pastoral counseling, and psychiatry.

CONCLUSIONS

This pilot study assessed the hypothesis that a creatively oriented positive human experience of therapeutic hypnosis could modulate gene expression on the molecular level. We documented changes in the expression of 15 early response genes within one hour that apparently initiated a further a further cascade of 77 genes 24 hours later. This proof-of-principle pilot study now requires cross validation with more subjects to document the validity and reliability of using DNA microarrays to assess our therapeutic protocol, The Creative Psychosocial Genomic Healing Experience, as a new approach for facilitating therapeutic hypnosis, psychotherapy, rehabilitation, meditation, and pastoral counselling.

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