Archival Report

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Coordinated Messenger RNA/MicroRNA Changes in Fibroblasts of Patients with Major Depression

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ABSTRACT

BACKGROUND: Peripheral biomarkers for major psychiatric disorders have been an elusive target for the last half a century. Dermal fibroblasts are a simple, relevant, and much underutilized model for studying molecular processes of patients with affective disorders, as they share considerable similarity of signal transduction with neuronal tissue.

METHODS: Cultured dermal fibroblast samples from patients with major depressive disorder (MDD) and matched control subjects ($n = 16$ pairs, 32 samples) were assayed for genome-wide messenger RNA (mRNA) expression using microarrays. In addition, a simultaneous quantitative polymerase chain reaction-based assessment of >1000 microRNA (miRNA) species was performed. Finally, to test the relationship between the mRNA-miRNA expression changes, the two datasets were correlated with each other.

RESULTS: Our data revealed that MDD fibroblasts, when compared with matched control subjects, showed a strong mRNA gene expression pattern change in multiple molecular pathways, including cell-to-cell communication, innate/adaptive immunity, and cell proliferation. Furthermore, the same patient fibroblasts showed altered expression of a distinct panel of 38 miRNAs, which putatively targeted many of the differentially expressed mRNAs. The miRNA-mRNA expression changes appeared to be functionally connected, as the majority of the miRNA and mRNA changes were in the opposite direction.

CONCLUSIONS: Our data suggest that combined miRNA-mRNA assessments are informative about the disease process and that analyses of dermal fibroblasts might lead to the discovery of promising peripheral biomarkers of MDD that could be potentially used to aid the diagnosis and allow mechanistic testing of disturbed molecular pathways.

Keywords: Biomarker, DNA microarray, Gene expression, Human fibroblasts, Major depression, miRNA

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There has been an intensive search for peripheral biomarkers of major psychiatric disorders for the last half a century. These efforts encompassed gene expression profiling of peripheral mononuclear cells (1,2); biochemical evaluations of serum (3), urine (4), saliva (5), and cerebrospinal fluid (6); gene association studies of DNA markers (7); and many other approaches. More recently, inducible pluripotent stem cells emerged as a very promising model for studying neuronal lineage disturbances across various disorders (8–10). Unfortunately, the complex diagnostic–phenotypic–genetic–etiologic heterogeneity continues (11) to provide significant obstacles for identifying highly specific and sensitive peripheral biomarkers of mental disorders.

Transcriptome profiling experiments of postmortem human brain tissue from subjects with major depressive disorder (MDD) suggest evidence of local inflammatory, apoptotic, oxidative stress and multiple other, nonbrain specific molecular processes (12–17). Emerging evidence argues that many of these changes might be, at least partially, driven by altered expression of microRNAs (miRNAs): miRNA levels change during stress in the brain of animal models of depression and in human postmortem brain of MDD subjects (18) and depressed suicide subjects (19).

Importantly, peripheral biomarker studies of MDD over the last several decades have revealed that peripheral growth factors, proinflammatory cytokines, endocrine factors, and metabolic markers all contribute to the disease pathophysiology (20). These combined data suggest that MDD is not only a disease of the central nervous system but also affects the whole body (21,22) and that peripheral cellular-molecular events are strongly correlated with the disease pathology in the central nervous system (23). Experimental data suggest that analyzing patient dermal fibroblasts is a simple, relevant, and much underutilized model for studying processes of signal transduction in patients with affective disorders (24–26). Dermal fibroblasts are easy to establish and maintain in culture without transformation, and the majority of confounding factors (e.g., life style or medication use) are virtually eliminated after several rounds of cell division. Furthermore, a recent study of fibroblasts obtained from MDD patients

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highlights the role that oxidative stress might play in the pathophysiology of MDD (25), which has been already well-established across multiple other research models and patient populations (27,28). Thus, analyzing dermal fibroblast cultures from patients can give us meaningful insights into the molecular effects of the combined genetic predisposition to the disorder.

It appears that biomarker panels hold a greater promise than single analyte molecules in aiding the diagnosis, monitoring of disease progression, or therapeutic response in MDD (20). As understanding nonneuronal changes in MDD can be informative of the overall disease pathophysiology (23), we performed a combined messenger RNA (mRNA)-miRNA profiling of dermal fibroblasts from patients with MDD and matched control subjects.

METHODS AND MATERIALS

Participants in the Study

The study was approved by the Vanderbilt University Institutional Review Board. Procedures for recruitment and diagnosis have been described previously (24,25). All participants were diagnosed with a current major depressive episode according to the Structured Clinical Interview for DSM-IV-TR (29) with an exclusion criteria of other primary Axis I DSM-IV diagnoses. A written informed consent was obtained from all participants before collecting skin biopsy samples. Sixteen pairs of subjects with MDD and healthy control subjects (CNTR) were matched by age, race, and sex. The average age of the MDD patients and CNTR were comparable (MDD = 34.9, CNTR = 35.2), as were the sex (12 female/4 male subjects in both groups) and race (12 white/4 African American), but there was a significant difference in body mass index at the time of skin biopsy (32.3 in MDD and 25.4 in CNTR, $p = .03$) (Table S1 in Supplement 1).

Human Dermal Fibroblasts

The skin biopsy was obtained from the lateral side of the upper arm (1 × 2 mm) according to a protocol previously described in detail (24,25). The sample was put into regular Dulbecco's Modified Eagle's Medium (MediaTech, Manassas, Virginia) without serum and processed the same day. Briefly, the biopsy sample was cut into several smaller pieces with scissors and incubated in trypsin and collagenase mix at 37°C for 1 hour. Regular medium was added (Dulbecco's modified Eagle's medium containing high glucose, L-glutamine, 10% fetal bovine serum [ThermoScientific HyClone, Logan, Utah], and penicillin/streptomycin solution [MediaTech]) and biopsy pieces with dissociated cells were pelleted by centrifugation. The supernatant was discarded. Biopsy pieces with cells were resuspended in fresh regular medium and transferred to 60-mm tissue culture plates. Cells were cultured in incubator at 37°C and 5% carbon dioxide concentration. Medium was changed three times a week. In about 2 to 3 weeks, the fibroblasts divided and became confluent. The fibroblasts were subcultured using .5% Trypsin-EDTA (Invitrogen, Carlsbad, California), as described elsewhere (30), and expanded for freezing in a liquid nitrogen cell repository or expanded for experiments. Selected fibroblasts from matching patient/control subject pairs were cultured simultaneously to ensure they grow under the same conditions. Cell growth and proliferation were checked regularly during the whole experiment. All cultured fibroblasts were less than passage 15. At the end of experiment, the fibroblasts were washed two times with ice-cold phosphate buffered saline, collected with cell scraper, pelleted by centrifugation, and frozen on dry ice and stored at −80°C.

mRNA Expression Analysis by Microarrays

Total RNA was isolated using the *mir*Vana miRNA Isolation Kit (Ambion, Foster City, California) and RNA quality was assessed by an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, California). Complementary DNA (cDNA) was generated using 2 μg of total RNA. cDNA synthesis, amplification, and labeling were performed using The Enzo Life Sciences Single-Round RNA Amplification and Biotin Labeling System (Enzo Life Sciences, Farmingdale, New York). Biotin labeled, fragmented amplified RNA (5 μg) was hybridized to a GeneChip HT HG-U133+ PM Array Plate (Affymetrix Inc, Santa Clara, California) at the Vanderbilt Microarray Shared Resource core facility.

Segmented images from each microarray were normalized and $\log_2$ transformed using GC-robust multi-array analysis (31). The normalized expression values were used in all analyses. Average expression values for each group (MDD and CNTR) were calculated for each gene probe. The magnitude of expression change was determined by the average logarithmic ratio ($ALR = mean_{MDD} - mean_{CNTR}$). Student paired and grouped two-tailed *t* tests were used to test the significance of the difference in gene expression (32–36). A gene was considered to be differentially expressed between MDD and CNTR when it met the dual criteria of ALR >.585 (50%) and both pairwise (ppval) and groupwise (gpval) *p* value < .05.

The differentially expressed genes were subjected to a two-way hierarchical clustering analysis based on Pearson correlation using GenePattern software (Broad Institute, Cambridge, Massachusetts) (37). Gene set enrichment analysis (GSEA) based on predefined gene classes was carried out with the GenePattern software (38). GSEA determines whether an a priori defined set of genes shows statistically significant, concordant differences between our subject groups based on the BioCarta defined molecular pathways (San Diego, California). GSEA calculates a normalized enrichment score, which reflects the degree to which a gene set is overrepresented in the ranked list of genes, and a nominal *p* value, which estimates the statistical significance of the enrichment score for a single gene set. BioCarta gene sets were considered differentially expressed at nominal *p* value < .05.

mRNA Data Validation by Quantitative Polymerase Chain Reaction

cDNA was generated with random primers using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California). Primers for 13 genes (heparin-binding EGF-like growth factor—*HBEGF*; major histocompatibility complex, class II invariant chain—*CD74*; major histocompatibility complex, class II, DP alpha 1—*HLA-DPA1*; glutathione S-transferase theta 1—*GSTT1*; major histocompatibility