Gene Set Enrichment Analysis (GSEA) of Upregulated Genes in Cocaine Addiction Reveals miRNAs as Potential Therapeutic Agents

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Abstract

Cocaine addiction is a global health problem that causes substantial damage to the health of addicted individuals around the world. Dopamine synthesizing (DA) neurons in the brain play a vital role in the addiction to cocaine. But the underlying molecular mechanisms that help cocaine exert its addictive effect have not been very well understood. Bioinformatics can be a useful tool in the attempt to broaden our understanding in this area. In the present study, Gene Set Enrichment Analysis (GSEA) was carried out on the upregulated genes from a dataset of DA neurons of post-mortem human brain of cocaine addicts. As a result of this analysis, 3 miRNAs have been identified as having significant influence on transcription of the upregulated genes. These 3 miRNAs hold therapeutic potential for the treatment of cocaine addiction.

Keywords

GSEA; Cocaine; Addiction; Enrichment Analysis; Bioinformatics; *in silico* research

Introduction

Cocaine addiction is a public health problem that spans the whole world. It is associated with various somatic, psychological, socio-economic, and legal complications [1], [2]. Cocaine addiction is a disorder which is chronic and relapsing and is characterized by compulsive drug-seeking and drug use[3]. Addiction to drugs in general is thought to be linked with long-term changes in neural gene expression through various epigenetic mechanisms. These mechanisms practically form a 'molecular memory' that helps retaining the drug-addicted condition [4]

Despite the involvement of diverse neural cell types and circuits in creating the effects of drugs of abuse, the most important role is played by the dopamine (DA)-synthesizing neurons of the ventral midbrain. DA neurons innervate widespread regions of the forebrain. Only 1 in 200,000 neurons of the human brain are part of the midbrain DA cells. Despite being relatively small in number among the neural cellular population, DA cells are significant in mediating both the acute rewarding effects of drugs of abuse and the conditioned responses to cues associated

with previous drug use [5]. On the other hand, after cessation of chronic drug abuse, different types of adverse consequences (e.g. anhedonia and dysphoria) may arise from the lack of DA neurotransmission [6].

Activation of the meso-cortico-limbic system through elevation of dopamine release in the nucleus accumbens is how almost all psychoactive drugs cause addiction in humans [7]–[9]. Dopamine release is involved in motivational, emotional, contextual, and affective information processing of behaviour and drug reinforcement mechanisms. Dopamine levels in the synapses in the meso-cortico-limbic system get raised when cocaine blocks the transporter that pumps the neurotransmitter out of the synapse into the presynaptic nerve terminal. From animal and human studies (including brain imaging by positron emission tomography; PET) it has been found that the increased dopamine transmission plays a major role to the reinforcing effects of cocaine [10].

Previously it was hypothesized that only a simple dysfunction of the meso-cortico-limbic dopaminergic system is responsible for all aspects of cocaine addiction but that has been proven wrong as cocaine blocks the serotonin and norepinephrine presynaptic transporters as well and increases in both synaptic dopamine and norepinephrine levels have been found to mediate the rewarding cocaine subjective experience ('high') [11], [12]. Cocaine also alters the level of other neurotransmitters such as glutamate, GABA, endocannabinoid, and corticotrophin-releasing hormone [13]–[16]. Interactions between these neurotransmitter systems modulate the reward, motivation, and memory systems in the brain [17], [18].

Due to the critical role played by DA neurons in addiction, shedding more light on drug-induced molecular changes in these cells has become crucial yet our understanding of the nature of these changes still remains far from complete [19]. For gaining new insight into the pathophysiology of complex disorders such as drug addiction, postmortem human brain can be a unique resource despite the challenges associated with its use [20].

In this study, upregulated genes in DA neurons from postmortem human brain were identified to shed more light on the molecular mechanism, pathways and the key players involved with cocaine addiction. For doing this, gene expression dataset GSE54839 was used [17].

Methods

Identification of Upregulated Genes from GSE54839

From the NCBI website GEO datasets were searched using the term "cocaine AND differential gene expression" and reference series GSE54839 was analyzed with GEO2R.

For GEO2R analysis two groups termed “Cocaine addiction” and “Control” were defined. Thirty samples belonged to each groups. Using the GEOquery [21] and limma R [22] packages from the Bioconductor project, GEO2R analysis was performed [23]. Top 250 differentially expressed genes were found. P values were adjusted using the Benjamini & Hochberg (false discovery rate) method [24]. Log2 transformation to the data was applied. R script used to perform the calculation was obtained from the R script tab.

Enrichment Analysis

Enrichment analysis of the Upregulated genes was carried out using

- ChEA2016 TFs [27]
- MiRTarBase 2017 [28]
- KEGG 2016 [29]

Results and Discussion

R script

```
# Version info: R 3.2.3, Biobase 2.30.0, GEOquery 2.40.0, limma 3.26.8
# R scripts generated  Tue Apr 17 00:24:53 EDT 2018

################################################################
#   Differential expression analysis with limma
library(Biobase)
library(GEOquery)
library(limma)

# load series and platform data from GEO

gset <- getGEO("GSE54839", GSEMatrix =TRUE, AnnotGPL=TRUE)
if (length(gset) > 1) idx <- grep("GPL6947", attr(gset, "names")) else idx <- 1
gset <- gset[[idx]]

# make proper column names to match toptable
fvarLabels(gset) <- make.names(fvarLabels(gset))

# group names for all samples
gsms <- "111000111000111000111000111000111000111000111000111000111000111000"
sml <- c()
for (i in 1:nchar(gsms)) { sml[i] <- substr(gsms,i,i) }

# log2 transform
ex <- exprs(gset)
qx <- as.numeric(quantile(ex, c(0., 0.25, 0.5, 0.75, 0.99, 1.0), na.rm=T))
LogC <- (qx[5] > 100) ||
        (qx[6]-qx[1] > 50 && qx[2] > 0) ||
        (qx[2] > 0 && qx[2] < 1 && qx[4] > 1 && qx[4] < 2)
```

```
if (LogC) { ex[which(ex <= 0)] <- NaN
  exprs(gset) <- log2(ex) }

# set up the data and proceed with analysis
sml <- paste("G", sml, sep="")    # set group names
fl <- as.factor(sml)
gset$description <- fl
design <- model.matrix(~ description + 0, gset)
colnames(design) <- levels(fl)
fit <- lmFit(gset, design)
cont.matrix <- makeContrasts(G1-G0, levels=design)
fit2 <- contrasts.fit(fit, cont.matrix)
fit2 <- eBayes(fit2, 0.01)
tT <- topTable(fit2, adjust="fdr", sort.by="B", number=250)

tT <- subset(tT, select=c("ID","adj.P.Val","P.Value","t","B","logFC","Gene.symbol","Gene.title"))
write.table(tT, file=stdout(), row.names=F, sep="\t")


###################################################################
#   Boxplot for selected GEO samples
library(Biobase)
library(GEOquery)

# load series and platform data from GEO

gset <- getGEO("GSE54839", GSEMatrix =TRUE, getGPL=FALSE)
if (length(gset) > 1) idx <- grep("GPL6947", attr(gset, "names")) else idx <- 1
gset <- gset[[idx]]

# group names for all samples in a series
gsms <- "111000111000111000111000111000111000111000111000111000111000"
sml <- c()
for (i in 1:nchar(gsms)) { sml[i] <- substr(gsms,i,i) }
sml <- paste("G", sml, sep="")  set group names

# order samples by group
ex <- exprs(gset)[ , order(sml)]
sml <- sml[order(sml)]
fl <- as.factor(sml)
labels <- c("cocain","control")

# set parameters and draw the plot
palette(c("#dfeaf4","#f4dfdf", "#AABBCC"))
dev.new(width=4+dim(gset)[[2]]/5, height=6)
par(mar=c(2+round(max(nchar(sampleNames(gset)))/2),4,2,1))
title <- paste ("GSE54839", '/', annotation(gset), " selected samples", sep ='')
boxplot(ex, boxwex=0.6, notch=T, main=title, outline=FALSE, las=2, col=fl)
legend("topleft", labels, fill=palette(), bty="n")
```

Enrichment Analysis of Upregulated Genes					
ChEA2016 TFs					
Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	RELA_24523406_ChIP-Seq_FIBROSARCOMA_Human	3.88E-14	2.34E-11	-1.74	53.67
2	ATF3_23680149_ChIP-Seq_GBM1-GSC_Human	4.98E-10	1.50E-07	-1.59	34.06
3	ESR1_21235772_ChIP-Seq_MCF-7_Human	0.0001396	0.008406	-3.12	27.69
4	CLOCK_20551151_ChIP-Seq_293T_Human	0.00003391	0.002268	-2.66	27.38
5	TRIM28_21343339_ChIP-Seq_HEK293_Human	0.001042	0.03136	-3.45	23.67
MiRTarBase 2017					
Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	hsa-miR-124-3p	4.38E-07	0.000524	-9.8	143.46
2	hsa-miR-16-5p	0.0001975	0.03449	-10.09	86.04
3	hsa-miR-34a-5p	6.19E-07	0.000524	-5.13	73.27
4	hsa-miR-17-5p	0.005434	0.1546	-7.95	41.48
5	hsa-miR-15a-5p	0.0008265	0.08314	-5.35	37.94
KEGG 2016					
Index	Name	P-value	Adjusted p-value	Z-score	Combined score
1	TNF signaling pathway_Homo sapiens_hsa04668	0.000001233	0.00018	-1.91	26.05
2	Influenza A_Homo sapiens_hsa05164	0.000002546	0.0001858	-1.94	25.03
3	AGE-RAGE signaling pathway in diabetic complications_Homo sapiens_hsa04933	0.00001095	0.0005328	-2	22.9
4	Herpes simplex infection_Homo sapiens_hsa05168	0.00003759	0.001372	-1.71	17.38
5	Cocaine addiction_Homo sapiens_hsa05030	0.0001009	0.002945	-1.71	15.71

For the upregulated genes:

From the ChEA2016 TFs database, RELA_24523406_ChIP-Seq_FIBROSARCOMA_Human was found to be the most significant transcription factor. Hsa-miR-124-3p was identified as the most

significant miRNA from the MiRTarBase 2017. From the KEGG 2016 pathway analysis, TNF signaling pathway_Homo sapiens_hsa04668 was found to be the most significant pathway mediated by the upregulated genes.

Precursor of the mature miRNA, hsa-miR-124-3p, namely the miR-124 is a small non-coding RNA molecule which has been fund in flies [30], nematode worms [29], mouse [28] and human [31]. Dicer enzyme processes the mature ~21 nucleotide mature miRNAs from hairpin precursor sequences. MiR-124 is the most abundant miRNA expressed in neuronal cells. The sequence for hsa-miR-124-3p is- 53 - uaaggcacgcggugaaugccaa - 74 [32]

Precursor of the mature miRNA, hsa-miR-16-5p namely the miR-16 family is vertebrate specific and its members have been predicted or discovered in a number of different vertebrate species. The sequence for hsa-miR-124-3p is- 14 - uagcagcacguaaauauuggcg - 35 [32]

Precursor of the mature miRNA, hsa-miR-34a-5p namely the miR-34 family gives rise to three major mature miRNAs. Members of the miR-34 family were discovered computationally at first [33] and verified experimentally later [34], [35]. The sequence for hsa-miR-34a-5p is- 22 - uggcagugucuuagcugguugu - 43 [32]

Role of miRNAs as important regulatory agents for gene expression is being considered as therapeutic means in various diseases. Unlike siRNAs, miRNA-targeted therapy is capable of influencing not only a single gene, but entire cellular pathways or processes. Mitigating the effects exerted by overexpression of malignant miRNAs is possible through the application of artificial antagonists such as oligonucleotides or other small molecules. It is also possible to supplement miRNAs through the use of synthetic oligonucleotides [36]. In the case of current study, the miRNAs which were found to influence the transcription of upregulated genes in cocaine addiction can be supplemented so that they can negatively regulate those genes and thus reduce the addictive effects.

Conclusion

From the Gene Set Enrichment Analysis, 3 miRNAs have been discovered to be significantly associated with the transcription of upregulated genes in cocaine addiction. Therefore we predict that these 3 miRNAs hold therapeutic promise against cocaine addiction. Further studies *in vitro* should be carried out in order to get more knowledge about the efficacies of these miRNAs in mitigating the effects of cocaine addiction.

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