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Article

TNF signaling pathway upregulation as a potential pharmaceutical target for cocaine-addicted individuals

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SUMMARY

Cocaine is a highly addictive stimulant that induces the buildup of dopamine in the brain, which over-stimulates the body's reward system. Overdose deaths related to cocaine have been steadily increasing since 2014, with existing addiction treatments having limited capabilities. Therefore, the purpose of this investigation was to analyze the differentially expressed genes related to cocaine addiction and the cellular pathways they are associated with to expand potential targets for pharmacological therapies.

We used the dataset GSE54839 from the National Center for Biotechnology Information (NCBI) database to investigate the RNA expression differences between groups of chronic cocaine abusers and drug-free subjects. In our analysis, we split 60 samples into 2 test groups of 30 from an originally triplicated dataset. We identified about 370 significantly expressed genes (*p-value* < 5 x 10^{-4}). We categorized these genes as upregulated or downregulated genes using String-dB. We performed further enrichment testing using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) analyses.

In this dataset, we identified the Tumor Necrosis Factor (TNF) KEGG pathway as the most prominently upregulated pathway in cocaine-addicted individuals. Because of this, we believe that TNF pathway proteins have the potential to be pharmaceutical targets for treating cocaine addiction. However, the existing medications that mediate TNF activity mainly target autoimmune diseases, thus not guaranteeing that a protein could directly address cocaine addiction. Future research should further characterize the TNF pathway's efficacy as a pharmaceutical target.

INTRODUCTION

In 2019, cocaine-related overdose deaths rose to 15,883 in the United States, with fatalities increasing since 2014 (1). Cocaine is a highly addictive drug that increases dopamine levels in the brain, which stimulates the body's reward system and feelings of pleasure, resulting in a "high." Cocaine produces this "high" by causing a buildup of dopamine in the synaptic cleft between neurons, which causes responding neurons to receive increased dopamine signals (2). Normally, dopamine is transported back from the synaptic cleft to its originating neuron after stimulating the dopamine receptors of the receiving neuron. However, cocaine binds to the dopamine transporters that mediate this return, interfering with the recycling process and generating dopamine buildup (3).

Cocaine intoxication has many short- and long-term side effects and can be detrimental to physical and mental health. Short-term effects usually appear instantaneously after one dose and include sensations of euphoria, alertness, hypersensitivity, and paranoia (2). Cocaine intoxication can also cause constricted blood vessels, irregular heart rate, and increased blood pressure (2). Long-term effects are more severe, such as reduced blood flow to the gastrointestinal tract, cardiovascular toxicity, and increased risk of stroke (4). The inherent danger of cocaine is that after individuals have repeated exposure to the drug, the body starts to adopt a dependency on the substance. After repeated use, the brain's reward system becomes less sensitive to dopamine to adjust to the increasing buildup in the synaptic space. Therefore, the brain needs an increasing amount of dopamine to reach the same "highs," potentially encouraging users to take higher doses of cocaine to compensate (5).

Due to the severity of cocaine addiction, individuals who seek treatment have multiple treatment options like cognitive behavioral therapy, contingency management, therapeutic communities, and community-based recovery groups, all of which are psychosocial procedures (2). However, considering the addictiveness of cocaine, these strategies are not always the most effective, with many cocaine abuse programs experiencing high drop-out rates and facilitating inadequate periods of abstinence for their patients (6). Furthermore, psychosocial procedures may be less successful due to the ongoing pandemic, with many programs like in-person group therapy transitioning to an online space or becoming obsolete due to social distancing guidelines (7). In fact, the isolation and stress of the COVID-19 pandemic have increased drug overdose deaths to 81,000 in the United States, the highest number of mortalities ever recorded in a year (8). The effect of COVID-19 on drug abuse emphasizes the need for an even more robust set of treatment options for cocaine-addicted individuals.

Previous studies on how genetics relates to cocaine

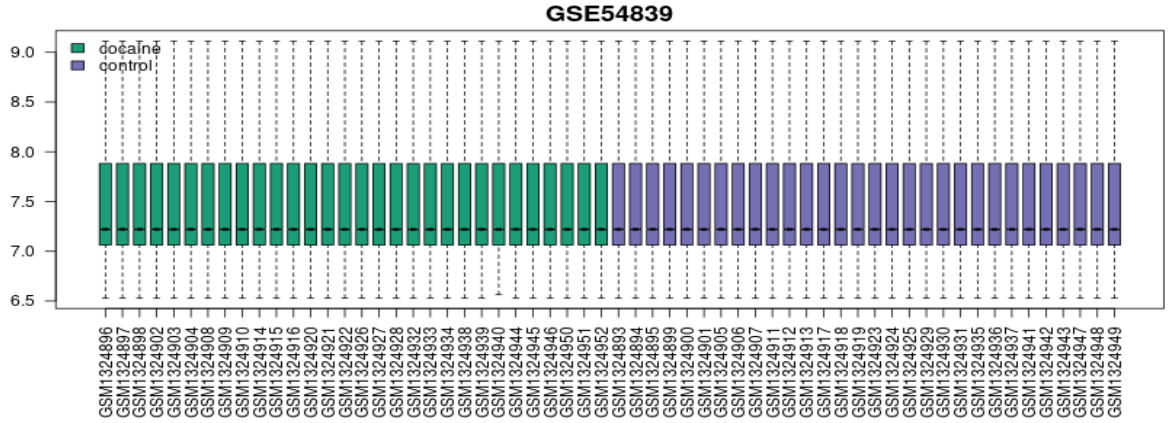

Figure 1: The gene expression from cocaine and control subjects did not contain any outliers. X-axis: the identification labels of the RNA mid-brain samples from NCBI dataset GSE54839. Y-axis: Normalized log-transformed values of microarray data representing gene expression level intensities. The value distribution graphic compares the relative gene expression levels of various mid-brain samples from dataset GSE54839. The green boxes represent the samples from cocaine addicted individuals and the purple boxes represent the samples from the control. Since the gene expression levels of all the samples were uniform, there were no outliers to exclude from the dataset (11).

abuse have shown that cocaine addiction is a heritable trait, with heritability around 65% in females and 79% in males (9). Specific genetic sequences like dopamine beta-hydroxylase and neuron-specific vascular protein genes have been linked to cocaine addiction (9). Furthermore, scientists have been experimenting with gene therapies, specifically injecting modified butyrylcholinesterase (BChE) genes into cocaine-addicted mice to produce enzymes that split the drug's molecules into harmless byproducts (10). These enzymes reduce the intensity of cocaine's euphoric effects and theoretically lower an addicted individual's chances of relapsing. However, because such therapies are unlikely to be developed into viable treatments due to technological and ethical concerns, other options are being explored to a further extent (9).

One such treatment option is the use of pharmaceuticals like dopamine agonists and glutamatergic medications to curb the addictiveness of cocaine (6). With dopamine agonists, the goal is for the medication to target the same receptors as cocaine, resulting in the same "high" with the reduced addictiveness of the agonist medication. However, many dopamine agonist trials addressing cocaine abuse have poor retention rates among participants, and these treatments have varying degrees of efficacy (6). On the other hand, glutamatergic medications decrease the activity of the brain's dopamine reward system - the drawback being that this effect might encourage cocaine-addicted individuals to take even higher doses to compensate for the reduced stimulation (6). Due to these issues, a pharmacological solution to cocaine addiction has yet to be approved for usage.

In this study, our goal was to assess the RNA expression differences related to cocaine abuse, and instead of identifying specific genetic sequences, we looked at the cellular pathways. We hypothesized that our analysis would reveal expression differences between cocaine-addicted and normal individuals. To investigate these expression differences, we analyzed the publicly accessible dataset GSE54839 from the National Center for Biotechnology Information (NCBI). The dataset came from a study that investigated the difference in gene expression regulating transcription, chromatin, and dopamine cell phenotypes in the human post-mortem midbrain between chronic cocaine abusers and drug-free subjects (11). The purpose of our investigation was to identify the enriched functions in this dataset's cocaine-addicted individuals based on whole-RNA expression patterns in an effort to expand the potential targets for current and future pharmacological addiction therapies.

RESULTS

Differential gene expression analysis

Due to a lack of time and resources to collect samples and conduct an experiment, we used publicly available dataset GSE54839 from NCBI. We chose this dataset because it had human (*Homo sapiens*) subjects and was conducted using expression profiling by array. When performing the analysis in the analysis software Gene Expression Omnibus (GEO2R), we divided the samples into two groups: the "cocaine" or "control" group. We chose these as our test groups because it was the only variable besides "cause of death" that changed throughout the samples. We decided not to choose "cause of death" due to its variability and lack of a control group. Since the samples in the value distribution were uniform, we concluded that outliers were not present (**Figure 1**).

Out of all the 48,760 genes in the dataset, we only identified and analyzed 369 in this paper. The rest of the genes did not meet the *p*-value cut-off of 5×10^{-4} and were deemed statistically insignificant. We then separated the significant genes into those upregulated or downregulated in the test group of cocaine-addict samples by their logFC values (**Figure 2**). Positive logFC values corresponded to upregulated genes and negative logFC values to downregulated genes. We did not use a logFC cut-off for this experiment because all the

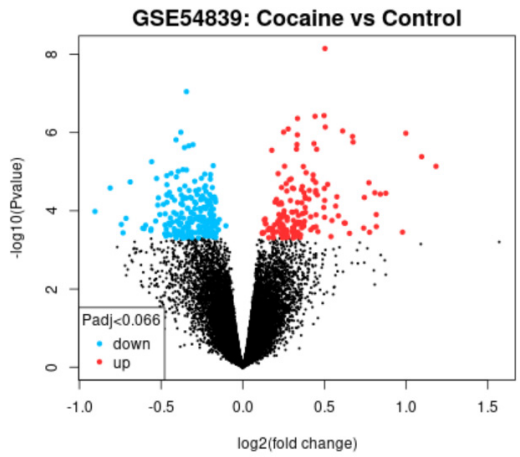

Figure 2: Upregulation and downregulation of significantly expressed genes. X axis: log2 fold change representing a ratio of a gene's expression levels in the mid-brain samples of cocaine-addicted individuals over individuals in the control. Y axis: -log10 *P-value* representing statistical significance. Volcano plot of the significantly expressed genes (*p-value* cut-off of 0.05) in the dataset GSE54839, allowing us to visualize the divergence of significantly upregulated and downregulated genes. Every dot represents a significant gene, with blue dots being downregulated genes and with red dots being upregulated genes in cocaine-addicted individuals. Since the volcano plot generation in our analysis software GEO2R depended on an adjusted *p-value*, the *p-value* cut-off of 0.05 was converted to an adjusted *p-value* cut-off of 0.066. We obtained this conversion by taking the adjusted *p-value* of the significantly expressed gene with the highest *p-value*, since *p-value* and adjusted *p-value* are proportional to each other. This ensured we visualized the same exact genes (11).

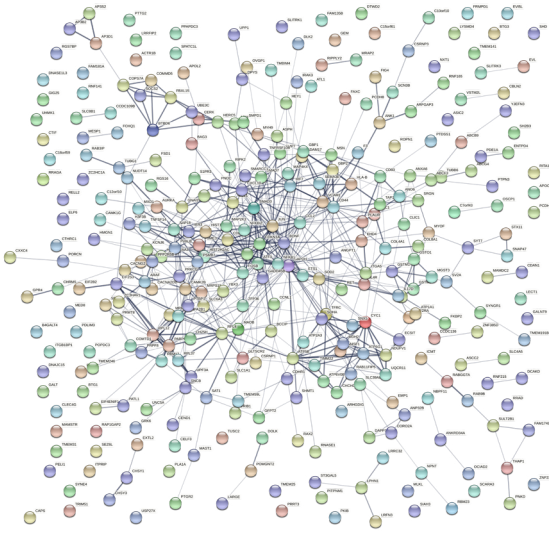

Figure 3: Protein-protein interactions of significantly expressed genes. String-dB, an analytical tool, mapped all the proteins of the significantly expressed genes of dataset GSE54839 (p-value cut-off of 0.05) and their interactions with each other. Each colored, circular node represents a protein that has been significantly expressed in cocaine-addicted individuals. The lines between each node indicate protein-protein interactions, with thicker lines suggesting a stronger interaction. The String-dB map, along with the program's analysis, gives insight into what proteins are related to each other and what pathways/biological processes they take part in (12).

significant genes had already been isolated (*p*-value < 5 x 10^{-4}). Of the 369 significant genes, 207 were downregulated, and 162 were upregulated. In addition, the logFC for downregulated genes ranged from -0.9046 to -0.1037. The logFC values for the upregulated genes ranged from 0.1194 to 1.1825.

Enrichment testing: String-dB and KEGG pathway analysis

We used the String-dB database to map out protein-protein interactions between all 369 differentially expressed genes of interest (**Figure 3**) (12). The most prominent Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, sorted from least to greatest false discovery rate, were cocaine addiction, amphetamine addiction, fluid shear stress, atherosclerosis, the oxytocin signaling pathway, herpes simplex infection, and the Tumor Necrosis Factor (TNF) signaling pathway (13-16). Cocaine and amphetamine addiction being the top KEGG pathways was expected given the nature of our samples and were not considered for our analysis. To better understand the regulated molecular pathways, we decided to investigate whether these prominent KEGG pathways would appear within solely upregulated or downregulated String-dB maps. We sought to identify a pathway in which a significant portion of its proteins was either enhanced or diminished, making it easier for potential pharmaceuticals to regulate such processes adequately.

In order to find prominent KEGG pathways, we mapped out the 162 upregulated and 207 downregulated genes in String-dB (**Figures 4A** and **4B**, respectively). Since the upregulated String-dB map yielded KEGG pathways with much lower false discovery rates, we decided to concentrate on the upregulated genes. Out of all the pathways, the TNF signaling pathway cluster had the lowest false discovery rate out of the enriched pathways of upregulated genes (**Table 1**). This gene cluster included 8 genes in a network of 108 genes (**Figure 5**). Due to its significance and low false discovery rate, we chose the TNF gene cluster for further analysis.

Enrichment Gene Ontology (GO) analysis:

When we conducted String-dB analysis on the upregulated genes, there were 370 Biological Processes/ Gene Ontology (GO) terms, with a *p*-value cut-off of < 5 x 10^{-4}. The top GO processes were Negative Regulation of Biological Processes, Response to Cytokine, Cellular Response to Cytokine Stimulus, and Regulation of Apoptotic Process (**Table 2**).

Common GO and KEGG pathway proteins:

The most notable GO process related to the KEGG TNF Signaling Pathway was cell surface signaling with 7 out of 8 genes shared with the pathway. The next most prominent was the cytokine GO process in which 6 out of 8 genes were shared between the pathway and GO process.

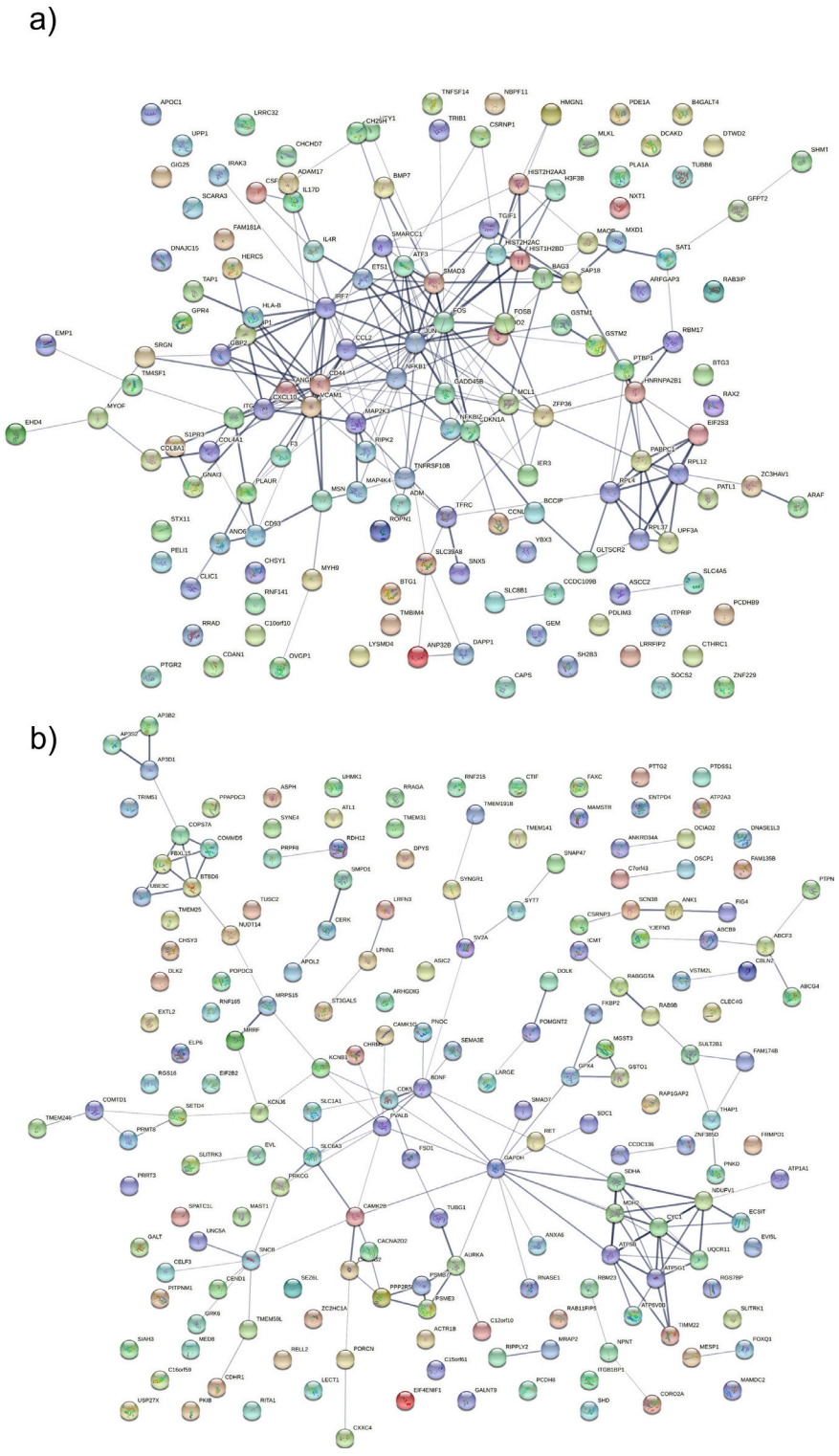

Figure 4: Protein-protein interactions of significantly (a) upregulated genes and (b) downregulated genes. String-dB, an analytical tool, mapped upregulated and downregulated proteins of significantly expressed genes from dataset GSE54839. A p-value cut-off of 0.05 determined whether a gene from the dataset was significantly expressed or not in cocaine-addicted mid-brain samples. We further separated the genes using logFC, with positive logFC values corresponding to upregulated genes and negative logFC values to downregulated genes. The protein symbols of these upregulated and downregulated genes were then inputted into String-dB, where separate (a) upregulated and (b) downregulated String maps were generated. Each colored, circular node represents a protein that has been significantly expressed in cocaine-addicted individuals. The lines between each node indicate protein-protein interactions, with thicker lines suggesting a stronger interaction. The String-dB map, along with the program's analysis, reveals differences in the protein interactions and pathways between upregulated and downregulated proteins (12).

Pathway	Description	False Discovery Rate
hsa04668	TNF` signaling pathway	0.00029
hsa05168	Herpes simplex infection	0.00029
hsa04657	IL-17 signaling pathway	0.00053
hsa04933	AGE-RAGE signaling pathway in diabetic complications	0.00053
hsa05200	Pathways in cancer	0.00053
hsa05166	HTLV-I infection	0.00073
hsa05030	Cocaine addiction	0.0013
hsa05210	Colorectal cancer	0.0013
hsa05164	Influenza A	0.0013
hsa05418	Fluid shear stress and atherosclerosis	0.0015

Table 1: The 10 KEGG pathways with the lowest false discovery rate from the significantly upregulated String-dB map. The left-most column lists the KEGG IDs of each pathway. The right-most column lists the false discovery rate of the specific pathway (12).

GO-Term	Description	False Discovery Rate
GO:0048519	Negative regulation of biological process	1.89e-10
GO:0034097	Response to cytokine	1.87e-09
GO:0071345	Cellular response to cytokine stimulus	4.21e-09
GO:0042981	Regulation of apoptotic process	1.17e-08
GO:0019221	Cytokine-mediated signaling pathway	1.36e-08
GO:0010941	Regulation of cell death	2.05e-08
GO:0007166	Cell surface receptor signaling pathway	5.04e-08
GO:0048523	Negative regulation of cellular process	4.04e-07
GO:0006950	Response to stress	7.28e-07
GO:0048518	Positive regulation of biological processes	7.87e-07

Table 2: The 10 biological GO processes with the lowest false discovery rate from the significantly upregulated String-dB map. The left-most column lists the GO-Term IDs of each pathway. The right-most column lists the false discovery rate of the specific pathway (12).

DISCUSSION

While the original investigators of this data set focused on the gene expression differences regulating transcription, chromatin, and dopamine cell phenotypes, we wanted to identify additional cellular functions affected by cocaine usage (11). Our analysis indicated that the TNF signaling pathway was upregulated in cocaine-addicted individuals. This pathway related to many biological processes, including cell surface signaling and cytokine response, as discovered in previous studies (17, 18). Preceding research also linked the TNF signaling pathway to neuronal degradation and inflammation (19, 20). Additionally, the expression of the TNF signaling pathway induced the degradation of certain nuclear receptor transcription factors, such as Retinoid-X-Receptors (RXRs) (19). These RXRs play a central role in dopaminergic signaling, dopamine-mediated locomotor activity, and reward processing in the brain's striatum (19). Thus, the TNF pathway may hold a crucial position in inhibiting a person's natural dopamine response, increasing the addictiveness of cocaine and dependency on the substance.

Identifying the TNF signaling pathway could lead to pharmaceuticals or other medical treatments that specifically target the pathway's proteins. These treatments would solve current psychosocial cocaine therapies' unreliability while providing patients with a wider array of recovery options. The most promising pharmaceutical medicines for addressing cocaine addiction revolve around dopamine agonists; however, targeting proteins in the TNF pathway that reduce dopamine-mediated activity and increase dopamine tolerance may prove more effective in decreasing a user's cocaine intake (5). Furthermore, since the TNF pathway is linked to negative effects such as neuronal degradation and inflammation, perhaps its proteins can be targeted to address such effects as well (19, 20).

Existing medicines like adalimumab, certolizumab pegol, etanercept, golimumab, and infliximab already target the TNF pathway and inhibit its activity, though they are mainly used to treat autoimmune diseases and largely target the immune system (21). These anti-TNF biologics can cause a few adverse effects, including a weakened immune system, a variable expression of TNF proteins, and an exacerbation of some diseases like multiple sclerosis and congestive heart failure (21). Although the majority of these medications do not cross the blood-brain barrier (BBB), research into the use of BBB-penetrating TNF inhibitors for the treatment of Alzheimer's disease is promising for the development of a pharmaceutical cocaine abuse therapy (21, 22). These BBB-penetrating medications would then eliminate the need for an intrathecal injection, which would involve an injection into the spine and would be an excessive solution to the already treatable problem of cocaine abuse.

Unfortunately, there are some limitations to this research on the potential uses of the TNF signaling pathway. The potential ramifications caused by pharmaceuticals manipulating the body's natural processes might not be worth the treatment benefits they provide to cocaine-addicted individuals, especially considering some of the side effects of existing TNF-targeting medications (21). There is also no guarantee that a pharmaceutical targeting TNF proteins will be specific enough for the sole use of treating cocaine addiction. Due to the robustness of the TNF pathway, it might be difficult to find a protein that specifically relates to cocaine usage. Another limitation to this paper is that we focused on one dataset from a single study. As a result, some other essential pathways and significant genes may have been overlooked. This research may also not be reproduced in a larger sample size. Furthermore, the original study had more thorough and flexible analysis methodologies, like being able to reduce

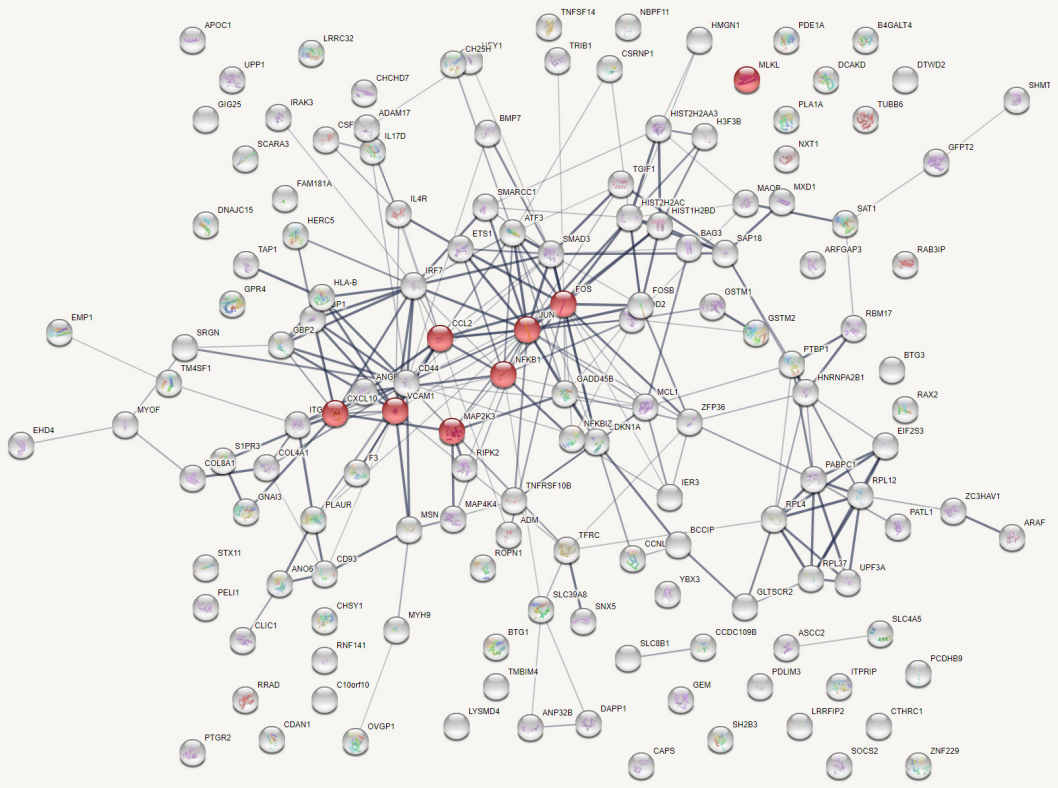

Figure 5: Upregulated TNF signaling pathway proteins. String-dB, an analytical tool, mapped the proteins from significantly upregulated genes in the dataset GSE54839. We separated the significantly upregulated genes using a p-value cut-off of 0.05 and a positive logFC requirement, then inputted their protein symbols into String-dB to generate a String map. Each grey, circular node represents a protein that has been significantly upregulated in cocaine-addicted individuals. The red nodes mark the 8 TNF signaling pathway proteins (CCL2, CXCL10, FOS, JUN, MAP2K3, MLKL, NFKB1, VCAM1). The lines between each node indicate protein-protein interactions, with thicker lines suggesting a stronger interaction. The String-dB map visualizes the TNF pathway proteins and their relations with each other and with proteins in other pathways (12).

variance through a matched pairs experimental design, while we had to adapt to GEO2R's more rigid analysis pipeline (11). Without some of these variance controls, our results may have been skewed. Future research may include analyzing larger groups of people, living samples in model organisms like mice, or non-midbrain samples to test the robustness of the TNF pathway. To ensure the viability of the TNF pathway as a pharmaceutical target, more research on whether such a pharmaceutical would be small enough to cross the BBB and affect the brain would have to be conducted.

Overall, our research demonstrated a genetic correlation between cocaine addiction and TNF signaling pathway upregulation. Our analysis with GO processes and KEGG pathways also reinforced previous scientific findings that the TNF pathway plays a role in cell surface signaling and cytokine response (17, 18). With these roles, the TNF pathway potentially inhibits a person's natural dopamine response, which increases their tolerance and likelihood of addiction (19). However, this correlation between cocaine addiction and the TNF signaling pathway suggests that possible medications targeting proteins in the pathway could be effective at treating cocaine addiction. A pharmaceutical treatment such as this not only provides more options for cocaine-addicted individuals but has the potential to improve upon the often-unreliable psychosocial programs employed today.

MATERIALS AND METHODS

Accessing Data

The GSE54839 dataset from the NCBI database was used for this research. In our analyses, 60 samples were divided into two test groups, with 30 samples in each test group. The differential gene expression analysis was conducted through the analytical software GEO2R, and samples were divided into either the "cocaine" or "control" group in GEO2R. The samples of the cocaine group were defined first in the software.

GEO2R, STRING, KEGG:

When performing the analysis with GEO2R, samples were divided into the "cocaine" or "control" group. Although the original dataset consisted of averaged triplicate samples, doing so was out of our technical capability in GEO2R, so we treated each triplicate as a sample. Therefore, we analyzed a total of 60 samples separated into two test groups of 30. After this, a modified Student T-Test was performed to assess differentially expressed genes between the "cocaine" and "control" group using GEO2R (11). Gene set enrichment testing was performed using String-dB, using its "multiple proteins" feature. We used genes identified from the t-test (*p*-value cut-off of 5 x 10^{-4}) as inputs. String-dB analyzes significant genes and visualizes their protein interactions in a String map. Based on the enriched genes expressed in the String map, the String-dB program generated lists of functional enrichments correlated to the chosen genes like GO processes and KEGG pathways. These lists gave us an idea of what physiological processes the enriched genes were a part of.

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REFERENCES

1. National Institute on Drug Abuse. "Overdose Death Rates." National Institute on Drug Abuse, National Institute on Drug Abuse, 25 Feb. 2021, www.drugabuse.gov/drug-topics/trends-statistics/overdose-death-rates. Accessed 16 Mar. 2021.
2. National Institute on Drug Abuse. "Cocaine DrugFacts." National Institute on Drug Abuse, National Institute on Drug Abuse, July 2019, www.drugabuse.gov/publications/drugfacts/cocaine. Accessed 17 Mar. 2021.
3. National Institute on Drug Abuse. "How Does Cocaine Produce Its Effects?" National Institute on Drug Abuse, National Institute on Drug Abuse, 11 June 2020, www.drugabuse.gov/publications/research-reports/cocaine/how-does-cocaine-produce-its-effects. Accessed 1 Jul. 2021.
4. National Institute on Drug Abuse. "What are the long-term effects of cocaine use?" National Institute on Drug Abuse, National Institute on Drug Abuse, 9 Jul. 2021, https://www.drugabuse.gov/publications/research-reports/cocaine/what-are-long-term-effects-cocaine-use Accessed 2 Jul. 2021.
5. National Institute on Drug Abuse. "The Neurobiology of Drug Addiction." National Institute on Drug Abuse, National Institute on Drug Abuse, Jan. 2007, www.drugabuse.gov/sites/default/files/1922-the-neurobiology-of-drug-addiction.pdf. Accessed 16 Mar. 2021.
6. Kampman, Kyle M. "The Treatment of Cocaine Use Disorder." Science Advances, vol. 5, no. 10, 16 Oct. 2019, doi:10.1126/sciadv.aax1532.
7. Oesterle, Tyler S., et al. "Substance Use Disorders and Telehealth in the COVID-19 Pandemic Era." Mayo Clinic Proceedings, vol. 95, no. 12, Dec. 2020, pp. 2709–2718., doi:10.1016/j.mayocp.2020.10.011.
8. Centers for Disease Control and Prevention (CDC). "Overdose Deaths Accelerating During COVID-19." CDC Newsroom, Centers for Disease Control and Prevention, 18 Dec. 2020, www.cdc.gov/media/releases/2020/p1218-overdose-deaths-covid-19.html. Accessed 17 Mar. 2021.
9. Pierce, Christopher R., et al. "Environmental, Genetic and Epigenetic Contributions to Cocaine Addiction." Neuropsychopharmacology, vol. 43, no. 7, 2018, pp. 1471–1480., doi:10.1038/s41386-018-0008-x.
10. National Institute of Drug Abuse. "Gene Transfer Therapy for Cocaine Addiction Passes Tests in Animals." NIDA Archives, National Institute of Drug Abuse, 14 Jan.2016,archives.drugabuse.gov/news-events/nida-notes/2016/01/gene-transfer-therapy-cocaine-addiction-passes-tests-in-animals. Accessed 21 Mar. 2021.
11. Bannon, M J, et al. "A Molecular Profile of Chronic Cocaine Abuse Includes Differential Expression of Genes Regulating Transcription, Chromatin and Dopamine Cell Phenotype." NCBI GEO, 1 Apr. 2014, www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54839.
12. STRING Consortium 2020. STRING, STRING Consortium 2020, 2020, string-db.org/cgi/input?sessionId=b4GXK8IErhpC&input_page_show_search=off. Accessed 7 Mar. 2021.
13. Kyoto Encyclopedia of Genes and Genomes (KEGG). KEGG PATHWAY Database, Kyoto Encyclopedia of Genes and Genomes (KEGG), 22 Feb. 2021, www.kegg.jp/kegg/pathway.html. Accessed 7 Mar. 2021.
14. Kanehisa, Minoru, and Susumu Goto. "KEGG: Kyoto Encyclopedia of Genes and Genomes." Nucleic Acids Research, vol. 28, no. 1, 2000, pp. 27–30., doi:https://doi.org/10.1093/nar/28.1.27.
15. Kanehisa, Minoru. "Toward Understanding the Origin and Evolution of Cellular Organisms." Protein Science, vol. 28, no. 11, Nov. 2019, pp. 1947–1951., doi:10.1002/pro.3715.
16. Kanehisa, Minoru, et al. "KEGG: Integrating Viruses and Cellular Organisms." Nucleic Acids Research, vol. 49, 2021, pp. D545–D551., doi:10.1093/nar/gkaa970.
17. Holbrook, Jonathan et al. "Tumour necrosis factor signalling in health and disease." F1000Research vol. 8 F1000 Faculty Rev-111. 28 Jan. 2019, doi:10.12688/f1000research.17023.1
18. Wajant, H, et al. "Tumor Necrosis Factor Signaling." Cell Death and Differentiation, vol. 10, 25 Mar. 2003, pp. 45–65., doi:https://doi.org/10.1038/sj.cdd.4401189.
19. Kovalevich, Jane, et al. "Cocaine Induces Nuclear Export and Degradation of Neuronal Retinoid X Receptor-γ via a TNF-α/JNK- Mediated Mechanism." Journal of

Neuroimmune Pharmacology, vol. 10, no. 1, 14 Jan. 2015, pp. 55–73., doi:10.1007/s11481-014-9573-x.
20. Cui, Changhai, et al. “Neuroimmune Mechanisms of Alcohol and Drug Addiction.” International Review of Neurobiology, 2014, pp. 1–12., doi:10.1016/b978-0-12-801284-0.00001-4.
21. Ping, Li, et al. “Drugs for Autoimmune Inflammatory Diseases: From Small Molecule Compounds to Anti-TNF Biologics .” Frontiers in Pharmacology , vol. 8, 2017, doi:10.3389/fphar.2017.00460.
22. Chang, Rudy, et al. “Blood–Brain Barrier Penetrating Biologic TNF-α Inhibitor for Alzheimer’s Disease.” Molecular Pharmaceutics, vol. 14, no. 7, 2017, pp. 2340–2349., doi:10.1021/acs.molpharmaceut.7b00200.

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